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(54) **MARKERS ASSOCIATED WITH SOYBEAN RUST RESISTANCE AND METHODS OF USE THEREFOR**

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A01H 5/10 (2018.01)
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CPC *A01H 1/04* (2013.01); *A01H 5/10* (2013.01); *C12N 15/8282* (2013.01); *C12Q 1/6895* (2013.01); *C12Q 2600/13* (2013.01); *C12Q 2600/156* (2013.01); *Y10T 436/143333* (2015.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

Methods for conveying soybean rust (SBR) resistance into non-resistant soybean germplasm are provided. In some embodiments, the methods include introgressing SBR resistance into a non-resistant soybean using one or more nucleic acid markers for marker-assisted breeding among soybean lines to be used in a soybean breeding program, wherein the markers are linked to and/or associated with SBR resistance. Also provided are single nucleotide polymorphisms (SNPs) associated with resistance to SBR; soybean plants, seeds, and tissue cultures produced by any of the disclosed methods; seed produced by the disclosed soybean plants; and compositions including amplification primer pairs capable of initiating DNA polymerization by a DNA polymerase on soybean nucleic acid templates to generate soybean marker amplicons.

7 Claims, 5 Drawing Sheets

Specification includes a Sequence Listing.

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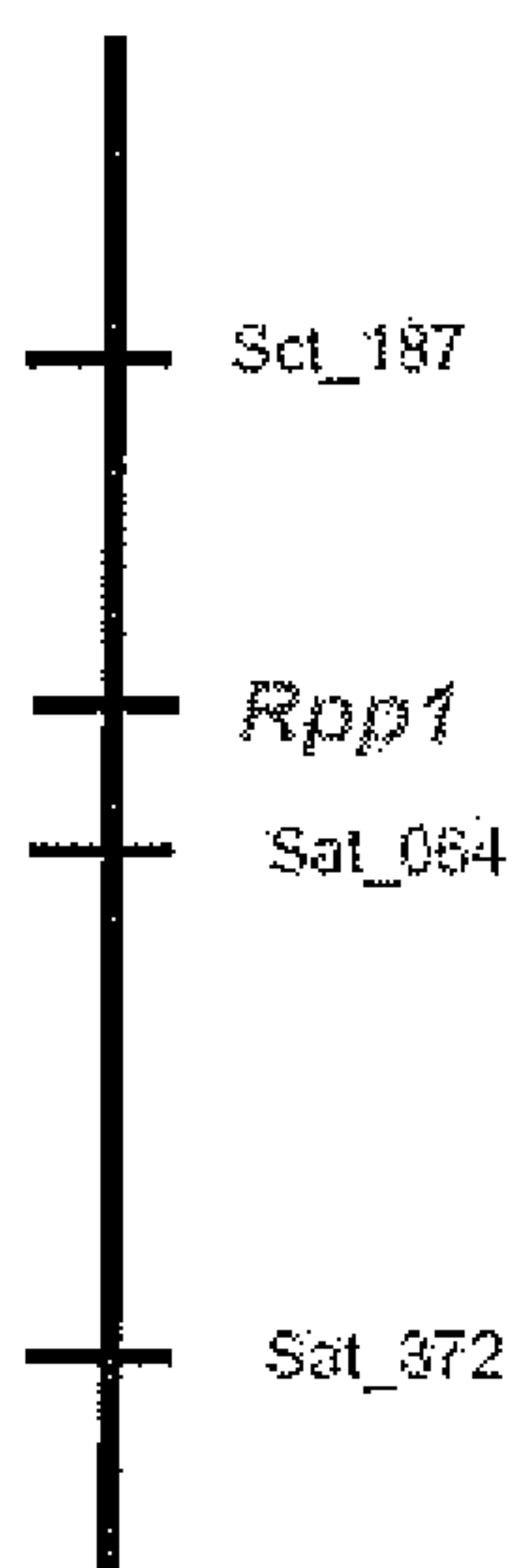
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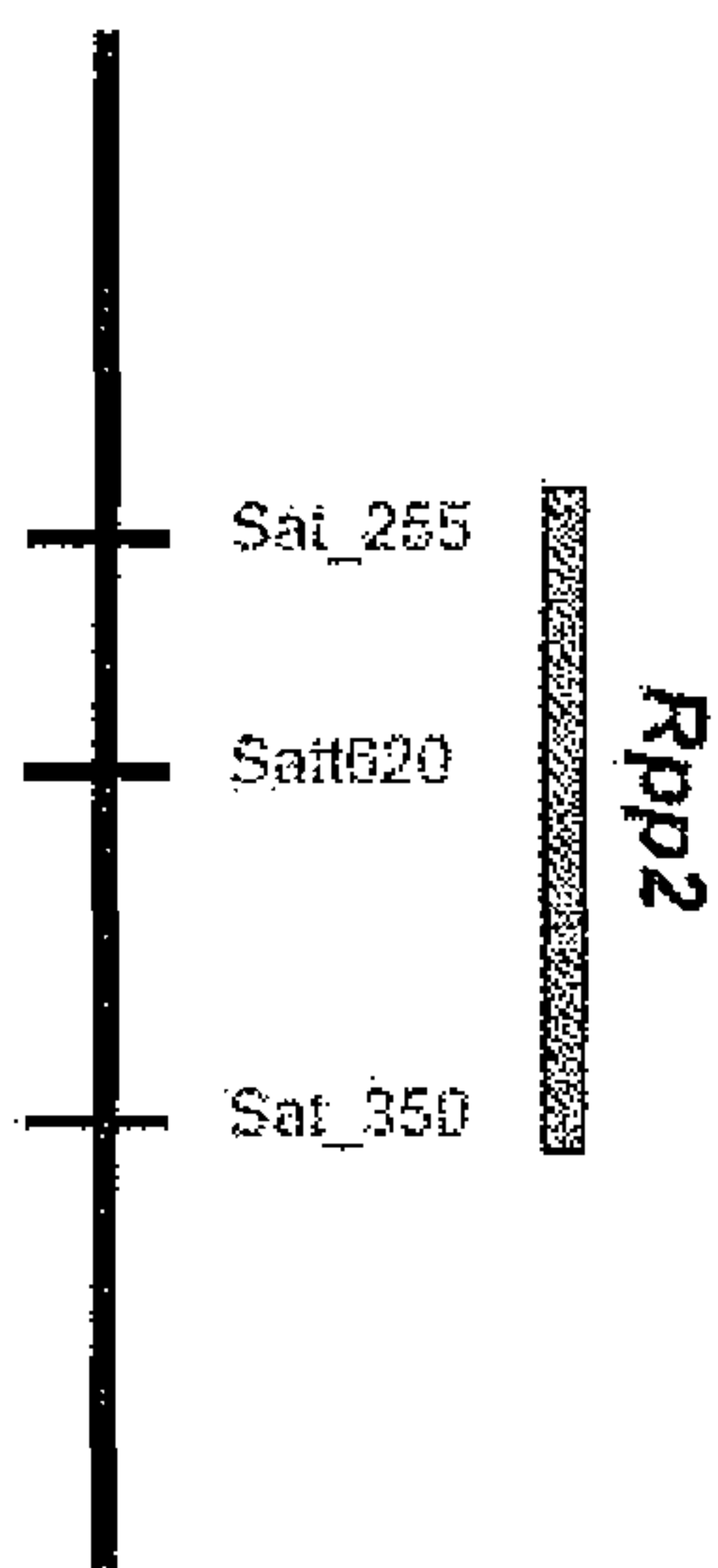
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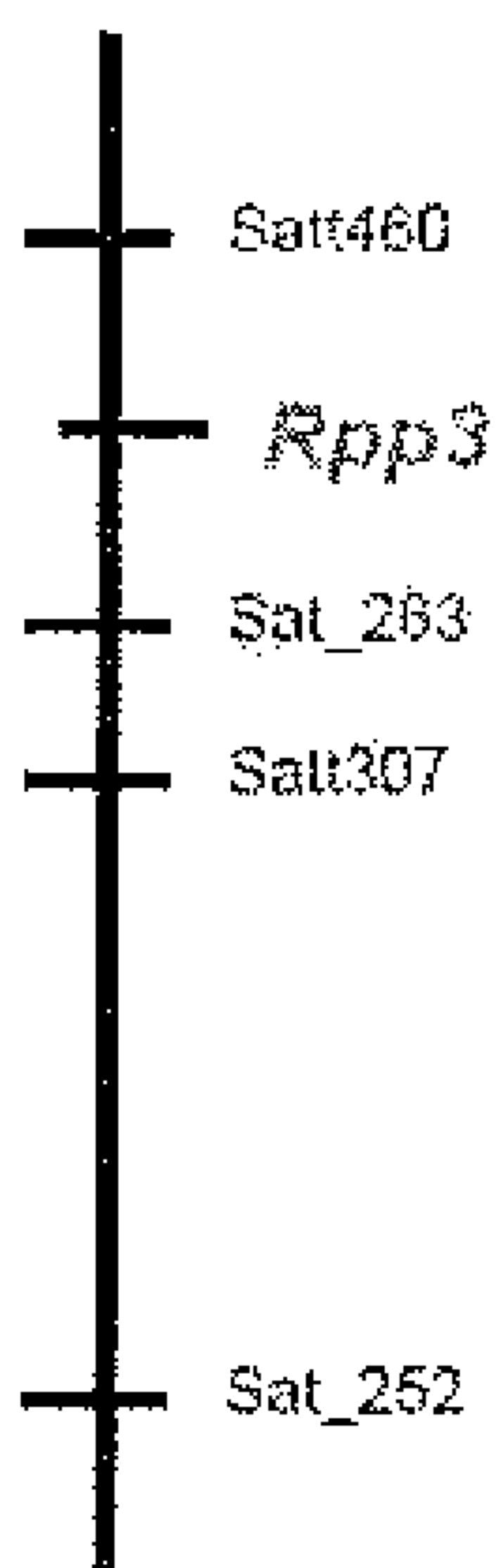
| MARKER NAME | MAP POSITION | MARKER TYPE |
|--------------------|---------------------|--------------------|
| Sct_187 | 100.374 | SSR |
| SEQ ID NO: 1 | 100.921 | SNP |
| Sat_064 | 101.823 | SSR |
| SEQ ID NO: 2 | 101.849 | SNP |
| SEQ ID NO: 3 | 103.63 | SNP |
| Sat_372 | 104.012 | SSR |

FIG. 1A



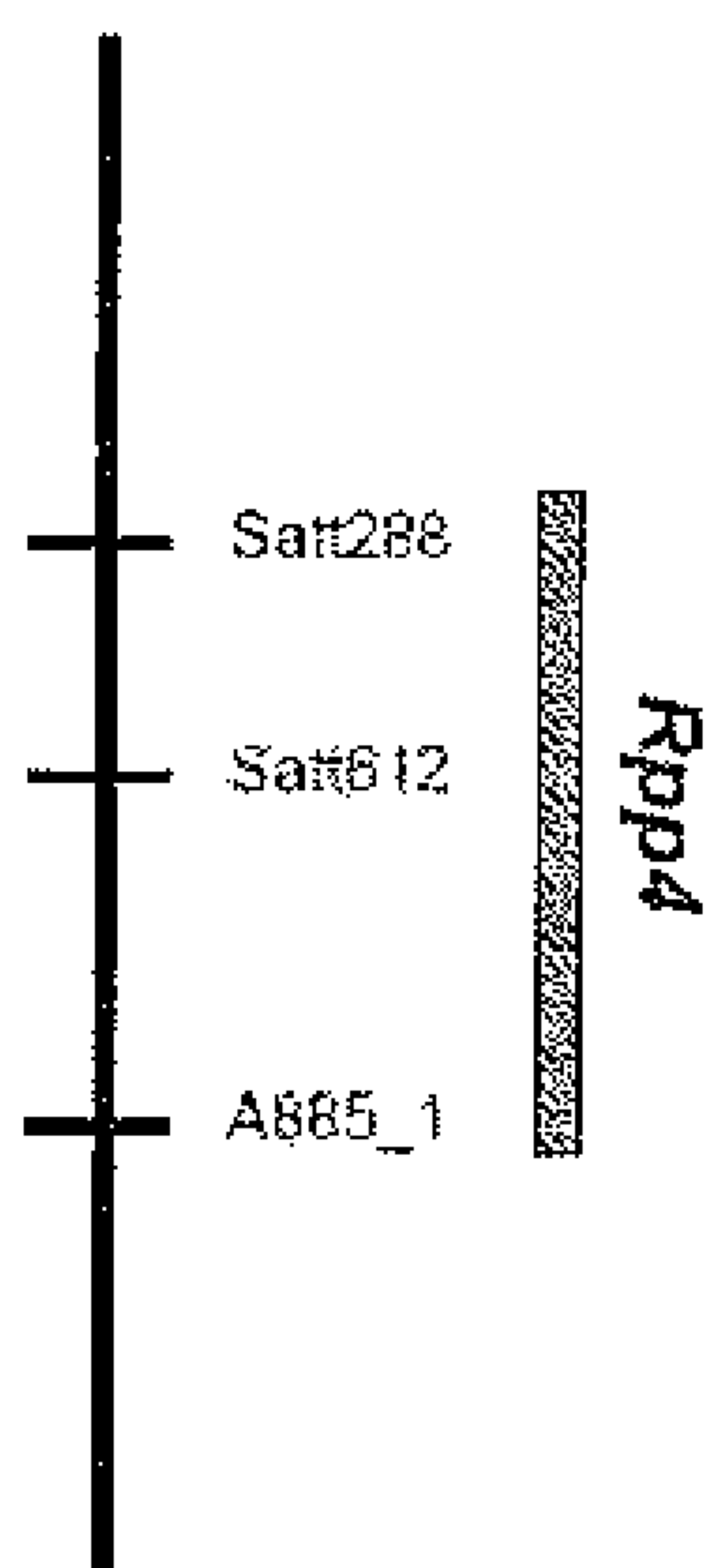
| MARKER NAME | MAP POSITION | MARKER TYPE |
|--------------|--------------|-------------|
| Sat_255 | 48.738 | SSR |
| SEQ ID NO: 4 | 49.858 | SNP |
| Satt620 | 53.705 | SSR |
| SEQ ID NO: 5 | 57.697 | SNP |
| Sat_350 | 60.717 | SSR |
| SEQ ID NO: 6 | 60.927 | SNP |

FIG. 1B



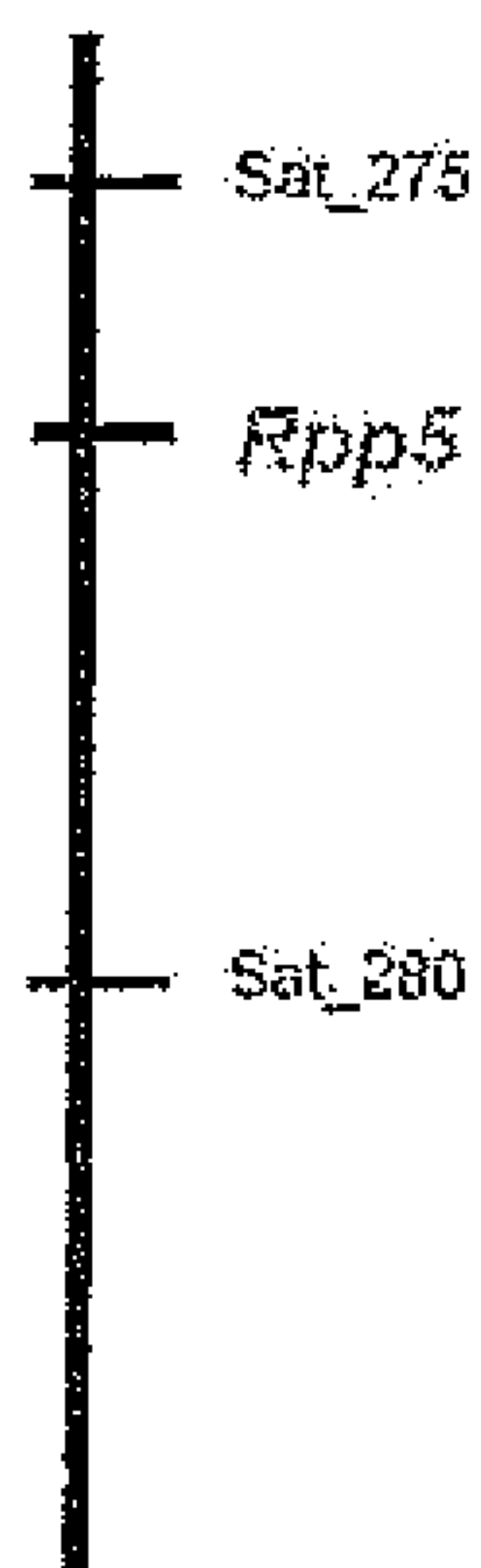
| MARKER NAME | MAP POSITION | MARKER TYPE |
|--------------|--------------|-------------|
| Satt460 | 106.991 | SSR |
| Sat_263 | 107.743 | SSR |
| Satt307 | 109.957 | SSR |
| SEQ ID NO: 7 | 115.74 | SNP |
| Sat_252 | 116.34 | SSR |
| SEQ ID NO: 8 | 117.9 | SNP |

FIG. 1C



| MARKER NAME | MAP POSITION | MARKER TYPE |
|----------------|--------------|-------------|
| Satt288 | 71.577 | SSR |
| Satt612 | 74.995 | SSR |
| SEQ. ID NO: 9 | 75.83 | SNP |
| SEQ. ID NO: 10 | 76.246 | SNP |
| A885_1 | 77.32 | RFLP |

FIG. 1D



| MARKER NAME | MAP POSITION | MARKER TYPE |
|---------------|--------------|-------------|
| Sat_275 | 32.488 | SSR |
| SEQ ID NO: 11 | 34.26 | SNP |
| SEQ ID NO: 12 | 34.27 | SNP |
| Sat_280 | 35.188 | SSR |
| SEQ ID NO: 13 | 38.8 | SNP |

FIG. 1 E

**MARKERS ASSOCIATED WITH SOYBEAN
RUST RESISTANCE AND METHODS OF USE
THEREFOR**

CROSS REFERENCE TO RELATED
APPLICATIONS

The following application is a continuation application of U.S. patent application Ser. No. 14/872,443 filed on Oct. 1, 2015 which is a divisional application of U.S. patent application Ser. No. 12/690,782 filed on Jan. 20, 2010, which itself claims the benefit of International Patent Application No. PCT/US2009/051003 filed on Jul. 17, 2009 which claims the benefit of U.S. Provisional Patent Application Ser. Nos. 61/081,989 and 61/153,495, filed Jul. 18, 2008 and Feb. 18, 2009, respectively. The disclosure of each of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING ELECTRONIC
FILING OF A SEQUENCE LISTING

A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled "72001-US-REG-D-NAT-1_Se-
qList_DIV_US_ST25", 34.2 kilobytes in size, generated on Sep. 1, 2015 and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated by reference into the specification for its disclosures.

TECHNICAL FIELD

The presently disclosed subject matter relates to markers associated with soybean rust (SBR) resistance and methods of use therefor. More particularly, the presently disclosed subject matter relates to screening soybean lines for resistance to SBR and for producing soybean lines with improved resistance to SBR, the methods involving genetic marker analysis.

BACKGROUND

Plant pathogens are known to cause massive damage to important crops, resulting in significant agricultural losses with widespread consequences for both the food supply and other industries that rely on plant materials. As such, there is a long felt need to reduce the incidence and/or impact of agricultural pests on crop production.

Soybean rust (SBR), which is caused by the obligate fungal pathogen *Phakopsora pachyrhizi* H. Sydow & Sydow, was first reported in Japan in 1902. By 1934, the pathogen was reported in several other Asian countries and in Australia. More recently, *P. pachyrhizi* infection has been reported in Africa, and has spread rapidly through the African continent.

In November 2004, *P. pachyrhizi* was first reported in the continental U.S., and the pathogen has now been reported in more than 300 U.S. counties, in Canada, and in Mexico. In 2007, approximately 0.5 million hectares of soybean were sprayed for SBR control in the U.S.

SBR has the potential to cause significant yield losses in the U.S., as indicated by fungicide trials in Georgia and Florida that reported yield losses of 30 to 33% in untreated control plots. In Brazil, the total yield loss in the 2006-2007 growing season due to SBR was estimated to be over U.S. \$2.26 billion with an average of 2.3 fungicide applications required per season. Yield losses up to 80% have been reported due to severe outbreaks of SBR, which result in early leaf drop that inhibits pod set. Consistent economic

losses in Brazil over the last several years due to severe SBR outbreaks have raised concerns regarding the potential impact of this disease in the United States. Soybean cultivars currently available commercially in the United States are all susceptible to SBR to some degree, and fungicide applications are currently employed to control the disease.

Therefore, soybean rust resistant cultivars are needed to reduce fungicide costs and yield losses due to SBR.

SUMMARY

The presently disclosed subject matter provides methods for conveying resistance to soybean rust (SBR) into non-resistant soybean germplasm. In some embodiments, the methods comprise introgressing SBR resistance into a non-resistant soybean using one or more nucleic acid markers for marker-assisted breeding among soybean lines to be used in a soybean breeding program, wherein the markers are linked to an SBR resistance locus selected from the group consisting of Rpp1, Rpp2, Rpp3, Rpp4, and Rpp5.

The presently disclosed subject matter also provides methods for reliably and predictably introgressing soybean rust (SBR) resistance into non-resistant soybean germplasm. In some embodiments, the methods comprise employing one or more nucleic acid markers for marker-assisted breeding among soybean lines to be used in a soybean breeding program.

The presently disclosed subject matter also provides methods for producing a soybean plant adapted for conferring resistance to soybean rust (SBR). In some embodiments, the methods comprise (a) selecting a first donor parental line possessing a desired SBR resistance and having at least one of the resistant loci selected from a locus mapping to Rpp1 and mapped by one or more of the markers SEQ ID NOs: 1-3; a locus mapping to Rpp2 and mapped by one or more of the markers SEQ ID NOs: 4-6; a locus mapping to Rpp3 and mapped by one or more of the markers SEQ ID NOs: 7 and 8; a locus mapping to Rpp4 and mapped by one or more of the markers SEQ ID NOs: 9 and 10; and a locus mapping to Rpp5 and mapped one or more markers SEQ ID NOs: 11-13; (b) crossing the first donor parent line with a second parental line in hybrid combination to produce a segregating plant population; (c) screening the segregating plant population for identified chromosomal loci of one or more genes associated with the resistance to SBR; and (d) selecting plants from the population having the identified chromosomal loci for further screening until a line is obtained which is homozygous for resistance to SBR at sufficient loci to give resistance to SBR.

The presently disclosed subject matter also provides methods for selecting a soybean rust (SBR) resistant soybean plant. In some embodiments, the methods comprise (a) genotyping one or more soybean plants with respect to one or more single nucleotide polymorphisms (SNPs); and (b) selecting a soybean plant that includes at least one resistance allele associated with the SNPs, thereby selecting an SBR resistant soybean plant.

In some embodiments, the presently disclosed methods comprise (a) isolating one or more nucleic acids from a plurality of soybean plants; (b) detecting in said isolated nucleic acids the presence of one or more marker molecules associated with SBR resistance, wherein said marker molecule is selected from the group consisting of SEQ ID NOs: 1-13; and (c) selecting a soybean plant comprising said one or more marker molecules, thereby selecting an SBR resistant soybean plant.

In some embodiments, the one or more nucleic acid markers are selected from the group consisting of SEQ ID NOs: 1-13, and informative fragments thereof. In some embodiments, the methods and compositions of the presently disclosed subject matter employ a marker molecule mapped within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 centiMorgans or less from a marker molecule selected from the group consisting of SEQ ID NOs: 1-13.

In some embodiments, the marker-assisted breeding comprises single nucleotide polymorphism (SNP) analysis. In some embodiments, the methods further comprise screening an introgressed soybean plant, or a cell or tissue thereof, for SBR resistance

In some embodiments of the presently disclosed methods, the at least one resistance allele is associated with an allele having an A at nucleotide 428 of SEQ ID NO: 1; a T at position 895 of SEQ ID NO: 2; a G at position 932 of SEQ ID NO: 2; a T at position 57 of SEQ ID NO: 3; a G at position 213 of SEQ ID NO: 4; a G at position 441 of SEQ ID NO: 4; an A at position 70 of SEQ ID NO: 5; a T at position 348 of SEQ ID NO: 5; an A at position 715 of SEQ ID NO: 6; a C at position 377 of SEQ ID NO: 7; a T at position 100 of SEQ ID NO: 8; a G at position 113 of SEQ ID NO: 8; a T at position 147 of SEQ ID NO: 9; a C at position 205 of SEQ ID NO: 10; an A at position 102 at SEQ ID NO: 11; A T at position 159 of SEQ ID NO: 12; and/or a G at position 357 of SEQ ID NO: 13.

The presently disclosed subject matter also provides soybean rust (SBR) resistant soybean plants, parts thereof (including but not limited to pollen, ovule, leaf, embryo, root, root tip, anther, flower, fruit, stem, shoot, seed; rootstock, protoplast, and callus), and progeny thereof, selected using the disclosed methods.

Thus, it is an object of the presently disclosed subject matter to provide methods for conveying SBR resistance into non-resistant soybean germplasm, which object is achieved in whole or in part by the presently disclosed subject matter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1E are genetic linkage maps depicting linkage groups and showing relative positions of various markers linked to Rpp1-Rpp5, respectively.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NOs: 1-13 are nucleotide sequences of the soybean genome comprising single nucleotide polymorphisms (SNPs) identified as being associated with the Rpp1 gene, the Rpp2 gene, the Rpp3 gene, the Rpp4 gene, and/or the Rpp5 gene as set forth in Table 1.

SEQ ID NOs: 14-81 are nucleotide sequences of oligonucleotide primers that can be employed to amplify and/or otherwise assay a subsequence of the soybean genome that is associated with the Rpp1-Rpp5 loci as also set forth in Table 1.

TABLE 1

| Amplification and Detection Primers | | | |
|-------------------------------------|--------------------------------|----------------------------------|-------------------------------|
| Rpp Gene | Genomic Subsequence SEQ ID NO: | Amplification Primer SEQ ID NOs. | Detection Primers SEQ ID NOs. |
| 1 | 1 | 14 and 15 | 16 and 17 |
| 1 | 2 | 18 and 19 | 20 and 21 |

TABLE 1-continued

| Amplification and Detection Primers | | | |
|-------------------------------------|--------------------------------|----------------------------------|-------------------------------|
| Rpp Gene | Genomic Subsequence SEQ ID NO: | Amplification Primer SEQ ID NOs. | Detection Primers SEQ ID NOs. |
| 1 | 2 | 22 and 23 | 24 and 25 |
| 1 | 3 | 26 and 27 | 28 and 29 |
| 2 | 4 | 30 and 31 | 32 and 33 |
| 2 | 4 | 34 and 35 | 36 and 37 |
| 2 | 5 | 38 and 39 | 40 and 41 |
| 2 | 5 | 42 and 43 | 44 and 45 |
| 2 | 6 | 46 and 47 | 48 and 49 |
| 3 | 7 | 50 and 51 | 53 and 53 |
| 3 | 8 | 54 and 55 | 56 and 57 |
| 3 | 8 | 58 and 59 | 60 and 61 |
| 4 | 9 | 62 and 63 | 64 and 65 |
| 4 | 10 | 66 and 67 | 68 and 69 |
| 5 | 11 | 70 and 71 | 72 and 73 |
| 5 | 12 | 74 and 75 | 76 and 77 |
| 5 | 13 | 78 and 79 | 80 and 81 |

SEQ ID NOs: 82-94 corresponds to subsequences of the preliminary assembly of the soybean (*Glycine max*) genomic sequence present in the Phytozome Database, which correspond to SEQ ID NOs: 1-13 as set forth in Table 2.

TABLE 2

| Locations of SEQ ID NOs: 1-13 in the Phytozome Database | | | |
|---|----------------------|--|------------------|
| SEQ ID NO: | Relevant Nucleotides | Nucleotide Positions in Phytozome Database | Percent Identity |
| 1 | 1-459 | 60469363-60469821 | 99.8 |
| 2 | 1-1101 | 60577757-60578858 | 99.1 |
| 3 | 23-424 | 60291869-60292270 | 98.8 |
| 4 | 1-470 | 27963429-27963894 | 99.6 |
| 5 | 1-489 | 30065112-30065600 | 99.4 |
| 6 | 1-794 | 30330727-30331520 | 98.7 |
| 7 | 1-568 | 46665732-46666299 | 99.3 |
| 8 | 1-503 | 41676695-41677197 | 98.8 |
| 9 | 1-769 | 56338383-56339144 | 97.9 |
| 10 | 1-513 | 55961511-55962023 | 99.6 |
| 11 | 1-281 | 33395967-33396246 | 99.3 |
| 12 | 36-916 | 33200392-33201274 | 97.4 |
| 13 | 5-479 | 35318068-35318542 | 99.4 |

DETAILED DESCRIPTION

The presently disclosed subject matter relates at least in part to the identification of several SNPs associated with SBR resistance in *Glycine* sp. Thus, provided herein are methods of conveying SBR resistance into non-resistant soybean germplasm, which employ one or more of the identified SNPs in various approaches.

All references listed below, as well as all references cited in the instant disclosure, including but not limited to all patents, patent applications and publications thereof, scientific journal articles, and database entries (e.g., GENBANK® database entries and all annotations available therein) are incorporated herein by reference in their entireties to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

I. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the presently disclosed subject matter belongs.

Following long-standing patent law convention, the articles “a”, “an”, and “the” refer to “one or more” when used in this application, including in the claims. For example, the phrase “a marker” refers to one or more markers. Similarly, the phrase “at least one”, when employed herein to refer to an entity, refers to, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, or more of that entity, including but not limited to whole number values between 1 and 100 and greater than 100.

Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about”. Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently disclosed subject matter.

As used herein, the term “about,” when referring to a value or to an amount of mass, weight, time, volume, concentration or percentage is meant to encompass variations of in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed method.

As used herein, the term “allele” refers to any of one or more alternative forms of a gene, all of which relate to at least one trait or characteristic. In a diploid cell, two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes, although one of ordinary skill in the art understands that the alleles in any particular individual do not necessarily represent all of the alleles that are present in the species. Since the presently disclosed subject matter relates to SNPs, it is in some instances more accurate to refer to a “haplotype” (i.e., an allele of a chromosomal segment) instead of “allele”. However, in such instances, the term “allele” should be understood to comprise the term “haplotype”.

As used herein, the phrase “associated with” refers to a recognizable and/or assayable relationship between two entities. For example, a trait, locus, QTL, SNP, gene, marker, phenotype, etc. is “associated with resistance” if the presence or absence of the trait, locus, QTL, SNP, gene, marker, phenotype, etc., influences an extent or degree of resistance (e.g., resistance to SBR). In some embodiments, an allele associated with resistance to SBR comprises an allele having an A at nucleotide 428 of SEQ ID NO: 1; a T at position 895 of SEQ ID NO: 2; a G at position 932 of SEQ ID NO: 2; a T at position 57 of SEQ ID NO: 3; a G at position 213 of SEQ ID NO: 4; a G at position 441 of SEQ ID NO: 4; an A at position 70 of SEQ ID NO: 5; a T at position 348 of SEQ ID NO: 5; an A at position 715 of SEQ ID NO: 6; a C at position 377 of SEQ ID NO: 7; a T at position 100 of SEQ ID NO: 8; a G at position 113 of SEQ ID NO: 8; a T at position 147 of SEQ ID NO: 9; a C at position 205 of SEQ ID NO: 10; an A at position 102 of SEQ ID NO: 11; A T at position 159 of SEQ ID NO: 12; and/or a G at position 357 of SEQ ID NO: 13.

As used herein, the term “backcross”, and grammatical variants thereof, refers to a process in which a breeder crosses a progeny individual back to one of its parents, for example, a first generation hybrid F1 with one of the parental genotypes of the F1 hybrid. In some embodiments,

a backcross is performed repeatedly, with a progeny individual of one backcross being itself backcrossed to the same parental genotype.

The term “chromosome” is used herein in its art-recognized meaning of the self-replicating genetic structure in the cellular nucleus containing the cellular DNA and bearing in its nucleotide sequence the linear array of genes.

As used herein, the terms “cultivar” and “variety” refer to a group of similar plants that by structural or genetic features and/or performance can be distinguished from other varieties within the same species.

As used herein, the term “gene” refers to a hereditary unit including a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic instruction for a particular characteristics or trait in an organism.

As used herein, the term “hybrid” in the context of plant breeding refers to a plant that is the offspring of genetically dissimilar parents produced by crossing plants of different lines or breeds or species, including but not limited to the cross between two inbred lines.

As used herein, the term “inbred” refers to a substantially homozygous individual or line.

As used herein, the phrase “informative fragment” refers to a nucleic acid molecule and/or its nucleotide sequence that allows for the proper identification of which allele of an allele set (e.g., an SNP) the nucleic acid molecule and/or the nucleotide sequence corresponds to. For example, whereas the SNP that corresponds to SEQ ID NO: 1 relates to an “A” or a “G” at position 428, an “informative fragment” of SEQ ID NO: 1 would be any sequence that comprises position 428 of SEQ ID NO: 1, thereby allowing the nucleotide that is present in that position to be determined.

As used herein, the terms “introgression”, “introgressed”, and “introgressing” refer to both a natural and artificial process whereby genomic regions of one species, variety, or cultivar are moved into the genome of another species, variety, or cultivar, by crossing those species. The process can optionally be completed by backcrossing to the recurrent parent.

As used herein, the term “linkage” refers to a phenomenon wherein alleles on the same chromosome tend to be transmitted together more often than expected by chance if their transmission was independent. Thus, in some embodiments two alleles on the same chromosome are said to be “linked” when they segregate from each other in the next generation less than 50% of the time, less than 25% of the time, less than 20% of the time, less than 15% of the time, less than 10% of the time, less than 5% of the time, less than 4% of the time, less than 3% of the time, less than 2% of the time, or less than 1% of the time. Thus, two loci are linked if they are within 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 centiMorgans (cM) of each other. For example, in some embodiments an SNP is linked to a marker if it is within 50, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 cM of the marker.

As used herein, the phrase “linkage group” refers to all of the genes or genetic traits that are located on the same chromosome. Within the linkage group, those loci that are close enough together can exhibit linkage in genetic crosses. Since the probability of crossover increases with the physical distance between loci on a chromosome, loci for which the locations are far removed from each other within a linkage group might not exhibit any detectable linkage in direct genetic tests. The term “linkage group” is mostly used to refer to genetic loci that exhibit linked behavior in genetic systems where chromosomal assignments have not yet been made. Thus, the term “linkage group” is synonymous with

the physical entity of a chromosome, although one of ordinary skill in the art will understand that a linkage group can also be defined as corresponding to a region of (i.e., less than the entirety) of a given chromosome.

As used herein, the term “locus” refers to a position that a given gene or a regulatory sequence occupies on a chromosome of a given species.

As used herein, the term “marker” refers to an identifiable position on a chromosome the inheritance of which can be monitored. In some embodiments, a marker comprises a known or detectable nucleic acid sequence.

In some embodiments, a marker corresponds to an amplification product generated by amplifying a *Glycine* sp. nucleic acid with two oligonucleotide primers, for example, by the polymerase chain reaction (PCR). As used herein, the phrase “corresponds to an amplification product” in the context of a marker refers to a marker that has a nucleotide sequence that is the same (allowing for mutations introduced by the amplification reaction itself) as an amplification product that is generated by amplifying *Glycine* sp. genomic DNA with a particular set of primers. In some embodiments, the amplifying is by PCR, and the primers are PCR primers that are designed to hybridize to opposite strands of the *Glycine* sp. genomic DNA in order to amplify a *Glycine* sp. genomic DNA sequence present between the sequences to which the PCR primers hybridize in the *Glycine* sp. genomic DNA. In some embodiments, a marker that “corresponds to” an amplified fragment is a marker that has the same sequence of one of the strands of the amplified fragment.

As used herein, the term “soybean” refers to a plant, or a part thereof, of the genus *Glycine* including, but not limited to *Glycine max*.

As used herein, the phrase “soybean-specific DNA sequence” refers to a polynucleotide sequence having a nucleotide sequence homology of in some embodiments more than 50%, in some embodiments more than 60%, in some embodiments more than 70%, in some embodiments more than 80%, in some embodiments more than 85%, in some embodiments more than 90%, in some embodiments more than 92%, in some embodiments more than 95%, in some embodiments more than 96%, in some embodiments more than 97%, in some embodiments more than 98%, and in some embodiments more than 99% with a sequence of the genome of the species *Glycine* that shows the greatest similarity to it. In the case of markers for any of the Rpp genes, a “soybean-specific DNA sequence” can comprise a part of the DNA sequence of a soybean genome that flanks and/or is a part of an Rpp gene sequence.

As used herein, the phrase “molecular marker” refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion and deletion mutations (INDEL), microsatellite markers (SSRs), sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location. A molecular marker “linked to” or “associated with” an Rpp gene as defined herein can thus refer to SNPs, insertion mutations, as well as more usual AFLP markers or any other type of marker used in the field.

As used herein, the phrase “nucleotide sequence homology” refers to the presence of homology between two polynucleotides. Polynucleotides have “homologous” sequences if the sequence of nucleotides in the two

sequences is the same when aligned for maximum correspondence. The “percentage of sequence homology” for polynucleotides, such as 50, 60, 70, 80, 90, 95, 98, 99 or 100 percent sequence homology, can be determined by comparing two optimally aligned sequences over a comparison window (e.g., about 20-200 contiguous nucleotides), wherein the portion of the polynucleotide sequence in the comparison window can include additions or deletions (i.e., gaps) as compared to a reference sequence for optimal alignment of the two sequences. Optimal alignment of sequences for comparison can be conducted by computerized implementations of known algorithms, or by visual inspection. Readily available sequence comparison and multiple sequence alignment algorithms are, respectively, the Basic Local Alignment Search Tool (BLAST®; Altschul et al. (1990) *J Mol Biol* 215:403-10; Altschul et al. (1997) *Nucleic Acids Res* 25:3389-3402) and ClustalX (Chenna et al. (2003) *Nucleic Acids Res* 31:3497-3500) programs, both available on the Internet. Other suitable programs include, but are not limited to, GAP, BestFit, PlotSimilarity, and FASTA, which are part of the Accelrys GCG Package available from Accelrys Software, Inc. of San Diego, Calif., United States of America.

As used herein, the term “offspring” plant refers to any plant resulting as progeny from a vegetative or sexual reproduction from one or more parent plants or descendants thereof. For instance, an offspring plant can be obtained by cloning or selfing of a parent plant or by crossing two parent plants and include selfings as well as the F1 or F2 or still further generations. An F1 is a first-generation offspring produced from parents at least one of which is used for the first time as donor of a trait, while offspring of second generation (F2) or subsequent generations (F3, F4, and the like) are specimens produced from selfings or crossings of F1s, F2s and the like. An F1 can thus be (and in some embodiments is) a hybrid resulting from a cross between two true breeding parents (the phrase “true-breeding” refers to an individual that is homozygous for one or more traits), while an F2 can be (and in some embodiments is) an offspring resulting from self-pollination of the F1 hybrids.

As used herein, the term “phenotype” refers to a detectable characteristic of a cell or organism, which characteristics are at least partially a manifestation of gene expression.

As used herein, the phrase “plant part” refers to a part of a plant, including single cells and cell tissues such as plant cells that are intact in plants, cell clumps, and tissue cultures from which plants can be regenerated. Examples of plant parts include, but are not limited to, single cells and tissues from pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, and seeds; as well as scions, rootstocks, protoplasts, calli, and the like.

As used herein, the term “population” refers to a genetically heterogeneous collection of plants sharing a common genetic derivation.

As used herein, the term “primer” refers to an oligonucleotide which is capable of annealing to a nucleic acid target and serving as a point of initiation of DNA synthesis when placed under conditions in which synthesis of a primer extension product is induced (e.g., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH). A primer (in some embodiments an extension primer and in some embodiments an amplification primer) is in some embodiments single stranded for maximum efficiency in extension and/or amplification. In some embodiments, the primer is an oligodeoxyribonucleotide. A primer is typically sufficiently long to prime the synthesis of extension and/or amplification

products in the presence of the agent for polymerization. The minimum lengths of the primers can depend on many factors, including, but not limited to temperature and composition (A/T vs. G/C content) of the primer.

In the context of amplification primers, these are typically provided as a pair of bi-directional primers consisting of one forward and one reverse primer or provided as a pair of forward primers as commonly used in the art of DNA amplification such as in PCR amplification.

As such, it will be understood that the term “primer”, as used herein, can refer to more than one primer, particularly in the case where there is some ambiguity in the information regarding the terminal sequence(s) of the target region to be amplified. Hence, a “primer” can include a collection of primer oligonucleotides containing sequences representing the possible variations in the sequence or includes nucleotides which allow a typical base pairing.

Primers can be prepared by any suitable method. Methods for preparing oligonucleotides of specific sequence are known in the art, and include, for example, cloning and restriction of appropriate sequences and direct chemical synthesis. Chemical synthesis methods can include, for example, the phospho di- or tri-ester method, the diethyl-phosphoramidate method and the solid support method disclosed in U.S. Pat. No. 4,458,066.

Primers can be labeled, if desired, by incorporating detectable moieties by for instance spectroscopic, fluorescence, photochemical, biochemical, immunochemical, or chemical moieties.

The PCR method is well described in handbooks and known to the skilled person. After amplification by PCR, target polynucleotides can be detected by hybridization with a probe polynucleotide which forms a stable hybrid with that of the target sequence under stringent to moderately stringent hybridization and wash conditions. If it is expected that the probes are essentially completely complementary (i.e., about 99% or greater) to the target sequence, stringent conditions can be used. If some mismatching is expected, for example if variant strains are expected with the result that the probe will not be completely complementary, the stringency of hybridization can be reduced. In some embodiments, conditions are chosen to rule out non-specific/adventitious binding. Conditions that affect hybridization, and that select against non-specific binding are known in the art, and are described in, for example, Sambrook & Russell (2001). *Molecular Cloning: A Laboratory Manual, Third Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., United States of America. Generally, lower salt concentration and higher temperature hybridization and/or washes increase the stringency of hybridization conditions.

Continuing, the term “probe” refers to a single-stranded oligonucleotide sequence that will form a hydrogen-bonded duplex with a complementary sequence in a target nucleic acid sequence analyte or its cDNA derivative.

As used herein, the terms “Rpp1”, “Rpp2”, “Rpp3”, “Rpp4”, and “Rpp5” refer to loci that have been associated with SBR resistance as defined by the markers defined herein. For the purposes of the instant disclosure, these loci are said to be present on *Glycine* linkage groups G, J, C2, G, and N, and linked to the markers depicted in FIGS. 1A-1E, respectively.

As used herein, the term “quantitative trait locus” (QTL; plural quantitative trait loci; QTLs) refers to a genetic locus (or loci) that controls to some degree a numerically representable trait that, in some embodiments, is continuously distributed. As such, the term QTL is used herein in its art-recognized meaning to refer to a chromosomal region

containing alleles (e.g., in the form of genes or regulatory sequences) associated with the expression of a quantitative phenotypic trait. Thus, a QTL “associated with” resistance to SBR refers to one or more regions located on one or more chromosomes and/or in one or more linkage groups that includes at least one gene the expression of which influences a level of resistance and/or at least one regulatory region that controls the expression of one or more genes involved in resistance to SBR. QTLs can be defined by indicating their genetic location in the genome of a specific *Glycine* sp. accession using one or more molecular genomic markers. One or more markers, in turn, indicate a specific locus. Distances between loci are usually measured by the frequency of crossovers between loci on the same chromosome. The farther apart two loci are, the more likely that a crossover will occur between them. Conversely, if two loci are close together, a crossover is less likely to occur between them. Typically, one centiMorgan (cM) is equal to 1% recombination between loci. When a QTL can be indicated by multiple markers, the genetic distance between the endpoint markers is indicative of the size of the QTL.

As used herein, the term “regenerate”, and grammatical variants thereof, refers to the production of a plant from tissue culture.

As used herein, the term “resistant” and “resistance” encompass both partial and full resistance to infection (e.g., infection by a pathogen that causes SBR). A susceptible plant can either be non-resistant or have lower levels of resistance to infection relative to a resistant plant. The term is used to include such separately identifiable forms of resistance as “full resistance”, “immunity”, “intermediate resistance”, “partial resistance”, and “hypersensitivity”.

As used herein, the phrase “stringent hybridization conditions” refers to conditions under which a polynucleotide hybridizes to its target subsequence, typically in a complex mixture of nucleic acids, but to essentially no other sequences. Stringent conditions are sequence-dependent and can be different under different circumstances. Exemplary guidelines for the hybridization of nucleic acids can be found in Tijssen (1993) in *Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier, New York, N.Y., United States of America; Ausubel et al. (1999) *Short Protocols in Molecular Biology* Wiley, New York, N.Y., United States of America; and Sambrook & Russell, 2001 (supra). Generally, stringent conditions are selected to be about 5-10° C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions are those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C. for long probes (e.g., greater than 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. Exemplary stringent hybridization conditions include: 50% formamide, 5×SSC, and 1% SDS, incubating at 42° C.; or 5×SSC, 1% SDS, incubating at 65° C.; with one or more washes in 0.2×SSC and 0.1% SDS at 65° C. For PCR, a temperature of about 36° C. is typical for low stringency amplification, although annealing temperatures can vary between about 32° C. and 48° C. (or higher) depending on primer length.

As used herein, the term “susceptible” refers to a plant having no resistance to the disease resulting in the plant being affected by the disease, resulting in disease symptoms. The term “susceptible” is therefore equivalent to “non-resistant”. Alternatively, the term “susceptible” can be employed in a relative context, in which one plant is considered “susceptible” because it is less resistant to a particular pathogen than is a second plant (which in the context of these terms in a relative usage, would be referred to as the “resistant” plant”).

II. General Considerations

Previous studies on host resistance to *P. pachyrhizi* have resulted in the identification of the four major resistance genes, Rpp1, Rpp2, Rpp3, and Rpp4, in soybean accessions PI 200492, PI 230970, PI 462312, and PI 459025B, respectively. Three of these genes (Rpp2, Rpp3, and Rpp4) confer a resistant reddish-brown (RB) colored lesion as opposed to the susceptible tan (TAN) colored lesion. Rpp1, on the other hand, confers resistance to some rust isolates. These four genes have been mapped on linkage groups (LGs) G, J, C2, and G, respectively. An RB lesion type resistance gene Rpp?(Hyuuga) from the Japanese cultivar “Hyuuga” has been mapped by to the same region on LG C2 as Rpp3. A fifth gene, Rpp5, was recently identified from PI200456 and mapped on LG N. With the availability of the 7× sequence coverage of the soybean genome made possible by efforts of the U. S. Department of Energy-Joint Genome Institute (DOE-JGI), Rpp1 has been fine mapped to a 23 kb region on scaffold 21 of LG G, and several markers close to this gene have been identified (Hyten et al. (2008) *Theor Appl Genet* 116:945-952).

Resistance conferred by Rpp1, Rpp2, Rpp3, Rpp4, and Rpp5 can be race-specific, and can be overcome by various *P. pachyrhizi* isolates. For example, resistance in soybean lines carrying either Rpp1 or Rpp3 genes failed in Brazil within two years of the establishment of the disease.

The presently disclosed subject matter provides in some embodiments soybean varieties that are resistant to SBR, methods for identifying soybean plants that carry desirable resistance genes, and methods for introducing such desirable resistance genes into soybeans.

III. Plant Breeding

The presently disclosed subject matter provides for better models for marker-assisted breeding (MAB). The presently disclosed subject matter therefore relates to methods of plant breeding and to methods to select plants, in particular soybean plants, particularly cultivated soybean plants as breeder plants for use in breeding programs or cultivated soybean plants for having desired genotypic or potential phenotypic properties, in particular related to producing valuable soybeans, also referred to herein as commercially valuable plants. Herein, a cultivated plant is defined as a plant being purposely selected or having been derived from a plant having been purposely selected in agricultural or horticultural practice for having desired genotypic or potential phenotypic properties, for example a plant obtained by inbreeding.

The presently disclosed subject matter thus also provides methods for selecting a plant of the genus *Glycine* exhibiting resistance towards SBR comprising detecting in the plant the presence of one or more resistance alleles as defined herein. In an exemplary embodiment of the presently disclosed methods for selecting such a plant, the method comprises providing a sample of genomic DNA from a soybean plant; and (b) detecting in the sample of genomic DNA at least one molecular marker associated with resistance to SBR. In

some embodiments, the detecting can comprise detecting one or more SNPs that are associated with resistance to SBR.

The providing of a sample of genomic DNA from a soybean plant can be performed by standard DNA isolation methods well known in the art.

The detecting of a molecular marker can in some embodiments comprise the use of one or more sets of primer pairs that can be used to produce one or more amplification products that are suitable markers for one of the SNPs. Such a set of primers can comprise, in some embodiments, nucleotide sequences as set forth in SEQ ID NOs: 14-81.

In some embodiments, the detecting of a molecular marker can comprise the use of a nucleic acid probe having a base sequence that is substantially complementary to the nucleic acid sequence defining the molecular marker and which nucleic acid probe specifically hybridizes under stringent conditions with a nucleic acid sequence defining the molecular marker. A suitable nucleic acid probe can for instance be a single strand of the amplification product corresponding to the marker. In some embodiments, the detecting of a molecular marker is designed to discriminate whether a particular allele of an SNP is present or absent in a particular plant.

The presently disclosed methods can also include detecting an amplified DNA fragment associated with the presence of a particular allele of an SNP. In some embodiments, the amplified fragment associated with a particular allele of an SNP has a predicted length or nucleic acid sequence, and detecting an amplified DNA fragment having the predicted length or the predicted nucleic acid sequence is performed such that the amplified DNA fragment has a length that corresponds (plus or minus a few bases; e.g., a length of one, two or three bases more or less) to the expected length as based on a similar reaction with the same primers with the DNA from the plant in which the marker was first detected or the nucleic acid sequence that corresponds (i.e., has a homology of in some embodiments more than 80%, in some embodiments more than 90%, in some embodiments more than 95%, in some embodiments more than 97%, and in some embodiments more than 99%) to the expected sequence as based on the sequence of the marker associated with that SNP in the plant in which the marker was first detected. Upon a review of the instant disclosure, one of ordinary skill in the art would appreciate that markers (e.g., SNP alleles) that are absent in resistant plants, while they were present in the susceptible parent(s) (so-called trans-markers), can also be useful in assays for detecting resistance among offspring plants.

The detecting of an amplified DNA fragment having the predicted length or the predicted nucleic acid sequence can be performed by any of a number of techniques, including but not limited to standard gel-electrophoresis techniques or by using automated DNA sequencers. The methods are not described here in detail as they are well known to the skilled person, although exemplary approaches are set forth in the EXAMPLES.

IV. Molecular Markers and SNPs

Molecular markers are used for the visualization of differences in nucleic acid sequences. This visualization can be due to DNA-DNA hybridization techniques after digestion with a restriction enzyme (e.g., an RFLP) and/or due to techniques using the polymerase chain reaction (e.g., STS, SSR/microsatellites, AFLP, and the like). In some embodiments, all differences between two parental genotypes segregate in a mapping population based on the cross of these parental genotypes. The segregation of the different markers

can be compared and recombination frequencies can be calculated. Methods for mapping markers in plants are disclosed in, for example, Glick & Thompson (1993) *Methods in Plant Molecular Biology and Biotechnology*, CRC Press, Boca Raton, Fla., United States of America; Zietkiewicz et al. (1994) *Genomics* 20:176-183.

The recombination frequencies of molecular markers on different chromosomes and/or in different linkage groups are generally 50%. Between molecular markers located on the same chromosome or in the same linkage group, the recombination frequency generally depends on the distance between the markers. A low recombination frequency typically corresponds to a low genetic distance between markers on a chromosome. Comparing all recombination frequencies results in the most logical order of the molecular markers on the chromosomes or in the linkage groups. This most logical order can be depicted in a linkage map. A group of adjacent or contiguous markers on the linkage map that is associated with an increased level of resistance to a disease; e.g., to a reduced incidence of acquiring the disease upon infectious contact with the disease agent and/or a reduced lesion growth rate upon establishment of infection, can provide the position of a locus associated with resistance to that disease.

The markers disclosed herein can be used in various aspects of the presently disclosed subject matter as set forth hereinbelow. Aspects of the presently disclosed subject matter are not to be limited to the use of the markers identified herein, however. It is stressed that the aspects can also make use of markers not explicitly disclosed herein or even yet to be identified. Other than the genetic unit "gene", on which the phenotypic expression depends on a large number of factors that cannot be predicted, the genetic unit "QTL" denotes a region of the genome that is directly related to a phenotypic quantifiable trait.

The markers provided by the presently disclosed subject matter can be used for detecting the presence of one or more SBR resistance alleles of the presently disclosed subject matter in a suspected SBR-resistant soybean plant, and can therefore be used in methods involving marker-assisted breeding and selection of SBR resistant soybean plants. In some embodiments, detecting the presence of a particular allele of an SNP of the presently disclosed subject matter is performed with at least one of the markers for the resistance loci defined herein. The presently disclosed subject matter therefore relates in another aspect to a method for detecting the presence of a particular allele associated with SBR resistance, comprising detecting the presence of a nucleic acid sequence of the SNP in a suspected SBR-resistant soybean plant, which presence can be detected by the use of the disclosed markers and oligonucleotides.

The nucleotide sequence of an SNP of the presently disclosed subject matter can for instance be resolved by determining the nucleotide sequence of one or more markers associated with the SNP and designing internal primers for the marker sequences that can be used to determine which allele of the SNP is present in the plant.

In embodiments of such methods for detecting the presence of an SNP in a suspected SBR-resistant soybean plant, the method can also comprise providing a oligonucleotide or polynucleotide capable of hybridizing under stringent hybridization conditions to a particular nucleic acid sequence of an SNP, in some embodiments selected from the SNPs disclosed herein, contacting the oligonucleotide or polynucleotide with genomic nucleic acid (or a fragment thereof, including, but not limited to a restriction fragment thereof) of a suspected SBR-resistant soybean plant, and determining the presence of specific hybridization of the

oligonucleotide or polynucleotide to the genomic nucleic acid (or the fragment thereof).

In some embodiments, the method is performed on a nucleic acid sample obtained from the suspected SBR-resistant soybean plant, although in situ hybridization methods can also be employed. Alternatively, one of ordinary skill in the art can design specific hybridization probes or oligonucleotides capable of hybridizing under stringent hybridization conditions to the nucleic acid sequence of the allele associated with SBR resistance and can use such hybridization probes in methods for detecting the presence of an SNP allele disclosed herein in a suspected SBR-resistant soybean plant.

V. Production of SBR-Resistant Soybean Plants

The presently disclosed subject matter also relates to methods for producing an SBR-resistant soybean plant comprising performing a method for detecting the presence of an allele associated with resistance to SBR in a donor soybean plant according to the presently disclosed subject matter as described above, and transferring a nucleic acid sequence comprising at least one allele thus detected, or an SBR resistance-conferring part thereof, from the donor plant to an SBR-susceptible recipient soybean plant. The transfer of the nucleic acid sequence can be performed by any of the methods described herein.

An exemplary embodiment of such a method comprises the transfer by introgression of the nucleic acid sequence from an SBR-resistant donor soybean plant into an SBR-susceptible recipient soybean plant by crossing the plants. This transfer can thus suitably be accomplished by using traditional breeding techniques. SBR-resistance loci are introgressed in some embodiments into commercial soybean varieties using marker-assisted selection (MAS) or marker-assisted breeding (MAB). MAS and MAB involves the use of one or more of the molecular markers for the identification and selection of those offspring plants that contain one or more of the genes that encode for the desired trait. In the context of the presently disclosed subject matter, such identification and selection is based on selection of SNP alleles of the presently disclosed subject matter or markers associated therewith. MAB can also be used to develop near-isogenic lines (NIL) harboring the QTL of interest, allowing a more detailed study of each QTL effect and is also an effective method for development of backcross inbred line (BIL) populations. Soybean plants developed according to these embodiments can advantageously derive a majority of their traits from the recipient plant, and derive SBR resistance from the donor plant.

As discussed hereinabove, traditional breeding techniques can be used to introgress a nucleic acid sequence encoding SBR resistance into an SBR-susceptible recipient soybean plant. In some embodiments, a donor soybean plant that exhibits resistance to SBR and comprising a nucleic acid sequence encoding SBR resistance is crossed with an SBR-susceptible recipient soybean plant that in some embodiments exhibits commercially desirable characteristics, such as, but not limited to, disease resistance, insect resistance, valuable nutritional characteristics, and the like. The resulting plant population (representing the F1 hybrids) is then self-pollinated and set seeds (F2 seeds). The F2 plants grown from the F2 seeds are then screened for resistance to SBR. The population can be screened in a number of different ways.

First, the population can be screened using a traditional disease screen. Such disease screens are known in the art. In some embodiments, a quantitative bioassay is used. Second, marker-assisted breeding can be performed using one or

more of the herein-described molecular markers to identify those progeny that comprise a nucleic acid sequence encoding for SBR resistance. Other methods, referred to herein above by methods for detecting the presence of an allele associated with SBR resistance, can be used. Also, marker-assisted breeding can be used to confirm the results obtained from the quantitative bioassays, and therefore, several methods can also be used in combination.

Inbred SBR-resistant soybean plant lines can be developed using the techniques of recurrent selection and backcrossing, selfing, and/or dihaploids, or any other technique used to make parental lines. In a method of recurrent selection and backcrossing, SBR resistance can be introgressed into a target recipient plant (the recurrent parent) by crossing the recurrent parent with a first donor plant, which differs from the recurrent parent and is referred to herein as the "non-recurrent parent". The recurrent parent is a plant that is non-resistant or has a low level of resistance to SBR and possesses commercially desirable characteristics, such as, but not limited to (additional) disease resistance, insect resistance, valuable nutritional characteristics, and the like. In some embodiments, the non-recurrent parent exhibits SBR resistance and comprises a nucleic acid sequence that encodes SBR resistance. The non-recurrent parent can be any plant variety or inbred line that is cross-fertile with the recurrent parent.

The progeny resulting from a cross between the recurrent parent and non-recurrent parent are backcrossed to the recurrent parent. The resulting plant population is then screened for the desired characteristics, which screening can occur in a number of different ways. For instance, the population can be screened using phenotypic pathology screens or quantitative bioassays as known in the art. Alternatively, instead of using bioassays, MAB can be performed using one or more of the hereinbefore described molecular markers, hybridization probes, or polynucleotides to identify those progeny that comprise a nucleic acid sequence encoding SBR resistance. Also, MAB can be used to confirm the results obtained from the quantitative bioassays. In some embodiments, the markers defined herein are suitable to select proper offspring plants by genotypic screening.

Following screening, the F1 hybrid plants that exhibit an SBR-resistant phenotype or, in some embodiments, genotype and thus comprise the requisite nucleic acid sequence encoding SBR resistance, are then selected and backcrossed to the recurrent parent for a number of generations in order to allow for the soybean plant to become increasingly inbred. This process can be performed for two, three, four, five, six, seven, eight, or more generations. In principle, the progeny resulting from the process of crossing the recurrent parent with the SBR-resistant non-recurrent parent are heterozygous for one or more genes that encode SBR resistance.

In general, a method of introducing a desired trait into a hybrid soybean variety can comprise:

- (a) crossing an inbred soybean parent with another soybean plant that comprises one or more desired traits, to produce F1 progeny plants, wherein the desired trait is SBR resistance;
- (b) selecting the F1 progeny plants that have the desired trait to produce selected F1 progeny plants, in some embodiments using molecular markers as defined herein;
- (c) backcrossing the selected progeny plants with the inbred soybean parent plant to produce backcross progeny plants;

- (d) selecting for backcross progeny plants that have the desired trait and morphological and physiological characteristics of the inbred soybean parent plant, wherein the selection comprises the isolation of genomic DNA and testing the DNA for the presence of at least one molecular marker for SBR resistance, in some embodiments as described herein;
- (e) repeating steps (c) and (d) two or more times in succession to produce selected third or higher backcross progeny plants;
- (f) optionally selfing selected backcross progeny in order to identify homozygous plants; and
- (g) crossing at least one of the backcross progeny or selfed plants with another soybean parent plant to generate a hybrid soybean variety with the desired trait and all of the morphological and physiological characteristics of hybrid soybean variety when grown in the same environmental conditions.

As indicated, the last backcross generation can be selfed in order to provide for homozygous pure breeding (inbred) progeny for SBR resistance. Thus, the result of recurrent selection, backcrossing, and selfing is the production of lines that are genetically homogenous for the genes associated with SBR resistance, and in some embodiments as well as for other genes associated with traits of commercial interest.

VI. SBR-Resistant Soybean Plants and Seeds

The development of a hybrid soybean variety in a soybean plant breeding program can, in some embodiments, involve three steps: (1) the selection of plants from various germplasm pools for initial breeding crosses; (2) the selfing of the selected plants from the breeding crosses for several generations to produce a series of inbred lines, which, individually breed true and are highly uniform; and (3) crossing a selected variety with an different variety to produce the hybrid progeny (F1). After a sufficient amount of inbreeding successive filial generations will merely serve to increase seed of the developed inbred. In some embodiments, an inbred line comprises homozygous alleles at about 95% or more of its loci.

A SBR-resistant soybean plant, or a part thereof, obtainable by a method of the presently disclosed subject matter is an aspect of the presently disclosed subject matter.

The SBR-resistant soybean plants of the presently disclosed subject matter, or part thereof, can be heterozygous or homozygous for the resistance traits (in some embodiments, homozygous). Although the SBR resistance loci of the presently disclosed subject matter, as well as resistance-conferring parts thereof, can be transferred to any plant in order to provide for an SBR-resistant plant, the methods and plants of the presently disclosed subject matter are in some embodiments related to plants of the genus *Glycine*.

The SBR-resistant soybean lines described herein can be used in additional crossings to create SBR-resistant plants. For example, a first SBR-resistant soybean plant of the presently disclosed subject matter can be crossed with a second soybean plant possessing commercially desirable traits such as, but not limited to, disease resistance, insect resistance, desirable nutritional characteristics, and the like. In some embodiments, this second soybean line is itself SBR-resistant. In some embodiments, this line is heterozygous or homozygous for one or more of the disclosed SBR resistance loci, in order for one or more of these traits to be expressed in the hybrid offspring plants.

Another aspect of the presently disclosed subject matter relates to a method of producing seeds that can be grown into SBR-resistant soybean plants. In some embodiments, the method comprises providing a SBR-resistant soybean

plant of the presently disclosed subject matter, crossing the SBR-resistant plant with another soybean plant, and collecting seeds resulting from the cross, which when planted, produce SBR-resistant soybean plants.

In some embodiments, the method comprises providing a SBR-resistant soybean plant of the presently disclosed subject matter, crossing the SBR-resistant plant with a soybean plant, collecting seeds resulting from the cross, regenerating the seeds into plants, selecting SBR-resistant plants by any of the methods described herein, self-pollinating the selected plants for a sufficient number of generations to obtain plants that are fixed for an allele associated with SBR-resistance in the plants, backcrossing the plants thus produced with soybean plants having desirable phenotypic traits for a sufficient number of generations to obtain soybean plants that are SBR-resistant and have desirable phenotypic traits, and collecting the seeds produced from the plants resulting from the last backcross, which when planted, produce soybean plants which are SBR-resistant.

VII. Other Applications

With the use of these SNPs for breeding new soybean lines, a system for developing germplasm that has more than one mode of action against the fungus is made possible. The use of this dual mode of action will assist in inhibiting the fungus from developing resistance. Additionally, a regime for inhibiting fungal resistance can include a number of different modes of action. The different modes of action in an inhibiting fungus method could be through germplasm or seed treatments or chemical applications. A system for inhibiting fungal resistance includes germplasm with one or more vertical resistant traits, and/or germplasm with one or more horizontal tolerance traits with the possible options of including a seed treatment that is active against the fungus, and/or an antifungal spray. Antifungal sprays or treatments can include known antifungal compounds for this fungus but can also include glufosinate or glyphosate sprays, separate or in combination which also have an antifungal affect.

The regime for inhibiting antifungal resistance is very important for the continued effectiveness of germplasm resistance and chemical activity. There are areas in which soybeans are presently produced which have fungal isolates that are no longer negatively affected by at least two of the known Rpp resistance alleles. The ability of these fungal isolates to evolve so quickly could render entire soybean growing areas unprofitable unless the embodiments of the presently disclosed subject matter that provide soybean seeds which are protected by the SNP selected traits for a dual mode of action plus the optional seed treatment, or chemical applications is implemented.

EXAMPLES

The following Examples provide illustrative embodiments. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently claimed subject matter.

Example 1

SNP Analysis

There are SSR markers which are associated with soybean rust resistant qualitative and quantitative genes Rpp1, Rpp2, Rpp3, Rpp4, and Rpp5. This SSR information was

employed to identify SNP markers that map to the regions of qualitative genes and quantitative trait loci (QTL) regions defined for each of the Rpp1, Rpp2, Rpp3, Rpp4, and Rpp5 genes. The identified SNPs were validated on soybean rust resistant and susceptible lines. Analyses indicated that these SNPs mapped more closely and showed better associations to the Rpp gene than did the SSRs. The information of validated SNPs for soybean rust is new and is used for appropriate breeding programs.

Example 2

SNP Genotyping Data

Molecular markers were identified for the Rpp1, Rpp2, Rpp3, Rpp4, and Rpp5 genes, and the resistance gene first identified in the Japanese cultivar, Hyuuga. Hyten et al., 2007 used PI200492 to map Rpp1 to linkage group (LG) G between the SSR markers Sct_187 and Sat_064. Rpp2 was mapped in PI230970 to the region on LG J between Sat_255 and Satt620. Rpp4 was mapped in PI459025 on LG G between SSR Satt288 and RFLP marker A885_1 (Silva et al., 2008, supra). The cultivar Hyuuga (PI506764) was once thought to contain Rpp3 (the resistance gene contained in PIs 462312 and 459025B), but it is now believed that there are 2 separate rust resistance genes located near each other on LG C2, flanked by SSRs Satt460 and Sat_263. Rpp5 was recently identified in several lines (PI200487, PI200526, PI471904, PI200456) by Garcia et al., 2008, and mapped to LG N in a region flanked by the SSRs Sat_275 and Sat_280. The approximate positions of these genes and various markers are depicted in FIGS. 1A-1E.

The soybean linkage map developed by scientists at the USDA and made publicly available in 2006 can be found through the website of the United States Department of Agriculture (USDA) and is discussed in Choi et al. (2007) *Genetics* 176:685-696. This map was used to locate the flanking SSRs associated with each rust resistance gene and to select SNPs that were mapped within and close to each region. The polymorphism information and genomic sequence on either side of the SNP was used to design PCR-based assays to detect each allele. The sequences with the SNP indicated were either submitted to the Applied Biosystems Inc. (ABI; Foster City, Calif., United States of America) Assay-by-Design service for creation of custom TAQMAN® (Applied Biosystems Inc., Foster City, Calif., United States of America) based assays, or assays were manually designed using the ABI software PRIMER EXPRESS®. Similarly, TAQMAN® assays can be designed using software available from Biosearch Technologies (Novato, Calif., United States of America).

A goal of the SNP assay was to be able to determine which polymorphism(s), or allele(s), is/are present in the genome of any given soybean line, and ultimately to permit the selection of preferred allele(s) (i.e., rust resistant gene(s)), in a marker-assisted breeding program. A total of 17 SNPs were identified; four for Rpp1, five for Rpp2, three for Rpp3 (Hyuuga), two for Rpp4, and three for Rpp5. A total of 18 screening panel DNAs isolated from resistant lines (PI547875; PI200492; PI594538A; PI368039; PI547878; PI230970; PI224270; PI462312; PI578457A; PI518772; PI628932; PI506764; PI547879; PI459025B; PI200456; PI200526; PI200487; and PI471904) were used for the assays.

Allelic Discrimination Assays

In allelic discrimination assays, a PCR assay included a forward and reverse primer and a specific, fluorescent, dye-labeled probe for each of two alleles for a given SNP. The probes contained different fluorescent reporter dyes (VIC®; Applied Biosystems, Inc., Foster City, Calif., United States of America; and 6-carboxyfluorescein-aminohexyl amidite (FAM), or N-TET-6-Aminohexanol (TET) and FAM) to differentiate the amplification of each allele. A non-fluorescent quencher on each probe suppressed the fluorescence until amplification by PCR. During PCR, each probe annealed specifically to complementary sequences between the forward and reverse primer sites. Taq polymerase then cleaved the probes that were hybridized to each allele. Cleavage separated the reporter dye from the quencher, which resulted in increased fluorescence by the reporter dye.

Thus, the fluorescent signals generated by PCR amplification indicated that one or both alleles were present in the sample. In addition to the non-fluorescent quencher, the probes also contained a minor groove binder at the 3' end which resulted in increased melting temperatures (T_m), thereby allowing high specificity with the use of shorter oligos. These probes therefore exhibited greater T_m differences when hybridized to matched and mismatched templates, which provided more accurate allelic discriminations. Probes of this type were manufactured at either ABI (MGB™ quencher) or Biosearch Technologies (BHQ-PLUS™ quencher). At the end of PCR thermal cycling, fluorescence of the two reporter dyes was measured on an ABI 7900. An increase in fluorescence for one dye only indicated homozygosity for the corresponding allele. An increase in both fluorescent signals indicated heterozygosity.

Exemplary starting lines and haplotypes determined using this method are presented in Tables 3-8. In Tables 3-8, “H”

indicates that the line was heterozygous at that position, and “-” indicates that the nucleotide at that position was not determined.

TABLE 3

| SNP Screening Panel | | | | |
|---------------------|---------------|----------------------------|-------------------|------------------|
| Line | Name | Origin | Source of Seed | Resistance |
| PI547875 | L85-2378 | Developed in Illinois, USA | GRIN ¹ | Rpp1 |
| PI200492 | | | GRIN | Rpp1 |
| PI594538A | | | GRIN | Rpp1-b |
| PI368039 | Tainung No. 4 | | GRIN | Rpp1 |
| PI547878 | L86-1752 | Developed in Illinois, USA | GRIN | Rpp2 |
| PI230970 | | | GRIN | Rpp2 |
| PI224270 | | | GRIN | Rpp2 |
| PI462312 | Ankur | | GRIN | Rpp3 |
| PI578457A | | | GRIN | Rpp3 |
| PI 518772 | | | GRIN | Rpp3 |
| PI 628932 | | | GRIN | Rpp3 |
| PI506764 | | | GRIN | Rpp? (Hyuuga) |
| PI547879 | L87-0482 | Developed in Illinois, USA | GRIN | Rpp4 |
| PI459025B | (Bing nan) | | GRIN | Rpp4 |
| PI200456 | | | GRIN | Rpp5 |
| PI200526 | | | GRIN | Rpp5 |
| PI200487 | Kinoshita | | GRIN | Rpp5 |
| PI471904 | L85-2378 | Developed in Illinois, USA | GRIN | Rpp5 |

¹Germplasm Resources Information Network, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, United States of America.

TABLE 4

| SNP Genotyping Data - Detailed for Rpp1 | | | | | | |
|---|---------------|------------------|-------------------|---------------|---------------|--------------|
| | | | SEQ ID NO: 1 | SEQ ID NO: 2a | SEQ ID NO: 2b | SEQ ID NO: 3 |
| | | | Linkage Group | | | |
| | | | G | G | G | G |
| | | | Map Position (cM) | | | |
| SPIRIT Material ID | ABBRC | LOCUS | 100.921 Rpp1 | 101.849 Rpp1 | 101.849 Rpp1 | 103.63 Rpp1 |
| PI547875 | L85-2378 | Rpp1 | A | G | T | T |
| PI200492 | | Rpp1 | A | G | T | T |
| PI594538A | | Rpp1-b | A | G | T | T |
| PI368039 | Tainung No. 4 | Rpp1 | A | G | T | T |
| PI547878 | L86-1752 | Rpp2 | G | A | C | C |
| PI230970 | | Rpp2 | G | G | C | C |
| PI224270 | | Rpp2 | G | G | C | C |
| PI462312 | Ankur | Rpp3 | G | A | C | C |
| PI578457A | | Rpp3 | A | — | T | C |
| PI 518772 | | Rpp3 | G | A | C | C |
| PI 628932 | | Rpp3 | G | A | C | C |
| PI506764 | | Rpp? (Hyuuga) | G | G | C | C |
| PI547879 | L87-0482 | Rpp4 | G | A | C | C |
| PI459025B | (Bing nan) | Rpp4 | G | G | T | C |
| PI200456 | | Rpp5 | G | G | T | C |
| PI200526 | | Rpp5 | H | G | T | C |
| PI200487 | Kinoshita | Rpp5 | A | G | T | C |
| PI471904 | | Rpp5 | A | G | T | C |

TABLE 5

| SNP Genotyping Data - Detailed for Rpp2 | | | | | | | | | |
|---|---------------|------------------|-------------------|----------------|----------------|----------------|----------------|--|--|
| | | | SEQ ID NO: 4a | SEQ ID NO: 4b | SEQ ID NO: 5a | SEQ ID NO: 5b | SEQ ID NO: 6 | | |
| | | | Linkage Group | | | | | | |
| | | | J | J | J | J | J | | |
| | | | Map Position (cM) | | | | | | |
| SPIRIT Material ID | ABBRC | LOCUS | 49.858 Rpp2 | 49.858 Rpp2 | 57.697 Rpp2 | 57.697 Rpp2 | 60.927 Rpp2 | | |
| PI547875 | L85-2378 | Rpp1 | G | G | C | T | G | | |
| PI200492 | | Rpp1 | G | G | C | T | A | | |
| PI594538A | | Rpp1-b | G | G | C | T | A | | |
| PI368039 | Tainung No. 4 | Rpp1 | G | G | C | T | A | | |
| PI547878 | L86-1752 | Rpp2 | G | G | C | T | G | | |
| PI230970 | | Rpp2 | G | G | A | T | A | | |
| PI224270 | | Rpp2 | G | G | A | A | A | | |
| PI462312 | Ankur | Rpp3 | G | G | C | T | H | | |
| PI578457A | | Rpp3 | G | G | C | T | A | | |
| PI 518772 | | Rpp3 | G | G | H | T | A | | |
| PI 628932 | | Rpp3 | G | G | C | T | H | | |
| PI506764 | | Rpp? (Hyuuga) | G | G | A | A | A | | |
| PI547879 | L87-0482 | Rpp4 | G | G | C | T | G | | |
| PI459025B | (Bing nan) | Rpp4 | G | G | C | T | A | | |
| PI200456 | | Rpp5 | G | G | A | T | G | | |
| PI200526 | | Rpp5 | G | G | H | H | A | | |
| PI200487 | Kinoshita | Rpp5 | G | G | A | A | A | | |
| PI471904 | | Rpp5 | G | G | A | A | A | | |

TABLE 6

| SNP Genotyping Data - Detailed for Rpp3 | | | | | | |
|---|------------------|------------------|------------------------------------|-----------------------------------|-----------------------------------|--|
| | | | SEQ ID NO: 7 | SEQ ID NO: 8a | SEQ ID NO: 8b | |
| | | | Linkage Group | | | |
| | | | C2 | C2 | C2 | |
| | | | Map Position (cM) | | | |
| SPIRIT Material ID | ABBRC | LOCUS | 115.71 Rpp3 and Rpp?(Hyuuga) | 117.9 Rpp3 and Rpp?(Hyuuga) | 117.9 Rpp3 and Rpp?(Hyuuga) | |
| PI547875 | L85-2378 | Rpp1 | T | T | G | |
| PI200492 | | Rpp1 | C | T | G | |
| PI594538A | | Rpp1-b | C | T | G | |
| PI368039 | Tainung No. 4 | Rpp1 | C | T | G | |
| PI547878 | L86-1752 | Rpp2 | T | T | G | |
| PI230970 | | Rpp2 | C | T | G | |
| PI224270 | | Rpp2 | C | C | A | |
| PI462312 | Ankur | Rpp3 | C | T | G | |
| PI578457A | | Rpp3 | C | T | G | |
| PI 518772 | | Rpp3 | T | C | A | |
| PI 628932 | | Rpp3 | C | T | G | |
| PI506764 | | Rpp? (Hyuuga) | C | C | A | |
| PI547879 | L87-0482 | Rpp4 | T | T | G | |
| PI459025B | (Bing nan) | Rpp4 | C | T | G | |
| PI200456 | | Rpp5 | C | T | G | |
| PI200526 | | Rpp5 | C | H | G | |
| PI200487 | Kinoshita | Rpp5 | C | T | G | |
| PI471904 | | Rpp5 | C | T | G | |

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TABLE 7

| SNP Genotyping Data - Detailed for Rpp4 | | | | | |
|---|-------------|---------------|-----------|-------------------|--------|
| SPIRIT | Material ID | ABBRC | LOCUS | SEQ ID | SEQ ID |
| | | | | NO: 9 | NO: 10 |
| | | | | Linkage Group | |
| | | G | G | Map Position (cM) | |
| | | | | 75.83 | 76.246 |
| | | | | Rpp4 | Rpp4 |
| | PI547875 | L85-2378 | Rpp1 | C | A |
| | PI200492 | | Rpp1 | T | C |
| | PI594538A | | Rpp1-b | T | C |
| | PI368039 | Tainung No. 4 | Rpp1 | T | C |
| | PI547878 | L86-1752 | Rpp2 | C | A |
| | PI230970 | | Rpp2 | T | C |
| | PI224270 | | Rpp2 | C | A |
| | PI462312 | Ankur | Rpp3 | T | A |
| | PI578457A | | Rpp3 | T | C |
| | PI 518772 | | Rpp3 | T | C |
| | PI 628932 | | Rpp3 | C | A |
| | PI506764 | | Rpp? | C | C |
| | | | (Hyyuuga) | | |
| | PI547879 | L87-0482 | Rpp4 | T | C |
| | PI459025B | (Bing nan) | Rpp4 | T | C |
| | PI200456 | | Rpp5 | T | A |
| | PI200526 | | Rpp5 | C | H |
| | PI200487 | Kinoshita | Rpp5 | T | C |
| | PI471904 | | Rpp5 | T | C |

TABLE 8

| SNP Genotyping Data - Detailed for Rpp5 | | | | | | |
|---|-------------|---------------|-----------|---------------|-------------------|--------|
| SPIRIT | Material ID | ABBRC | LOCUS | SEQ ID | SEQ ID | SEQ ID |
| | | | | NO: 11 | NO: 12 | NO: 13 |
| | | | | Linkage Group | | |
| | | N | N | N | Map Position (cM) | |
| | | | | 34.26 | 34.27 | 38.8 |
| | | | | Rpp5 | Rpp5 | Rpp5 |
| | PI547875 | L85-2378 | Rpp1 | A | T | A |
| | PI200492 | | Rpp1 | G | A | G |
| | PI594538A | | Rpp1-b | A | T | G |
| | PI368039 | Tainung No. 4 | Rpp1 | A | T | G |
| | PI547878 | L86-1752 | Rpp2 | A | T | G |
| | PI230970 | | Rpp2 | A | T | A |
| | PI224270 | | Rpp2 | A | T | G |
| | PI462312 | Ankur | Rpp3 | G | A | G |
| | PI578457A | | Rpp3 | — | H | G |
| | PI 518772 | | Rpp3 | H | H | H |
| | PI 628932 | | Rpp3 | A | T | A |
| | PI506764 | | Rpp? | A | T | G |
| | | | (Hyyuuga) | | | |
| | PI547879 | L87-0482 | Rpp4 | A | T | G |
| | PI459025B | (Bing nan) | Rpp4 | A | T | A |
| | PI200456 | | Rpp5 | A | T | G |
| | PI200526 | | Rpp5 | G | H | G |

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 94

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 <211> LENGTH: 459
 <212> TYPE: DNA

24

TABLE 8-continued

| SNP Genotyping Data - Detailed for Rpp5 | | | | | | |
|---|-------------|-----------|-------|---------------|-------------------|--------|
| SPIRIT | Material ID | ABBRC | LOCUS | SEQ ID | SEQ ID | SEQ ID |
| | | | | NO: 11 | NO: 12 | NO: 13 |
| | | | | Linkage Group | | |
| | | N | N | N | Map Position (cM) | |
| | | | | 34.26 | 34.27 | 38.8 |
| | | | | Rpp5 | Rpp5 | Rpp5 |
| | PI200487 | Kinoshita | Rpp5 | A | T | G |
| | PI471904 | | Rpp5 | A | T | A |

Example 4

TAQMAN® Validation

To validate TAQMAN® allelic discrimination assays for association with disease resistance or tolerance, plants were selected based on their known phenotypic status and compared to the genotype at the specific SNP location. DNA isolated from leaf tissue of seedlings 7-10 days after planting was diluted in TE buffer and stored at 4° C. until used in PCR reactions as described below.

PCR was set up in 5 µl final volumes according to the following formula:

| Reagent | Stock concentration | Per rxn (µl) | For 96 samples (µl) | Final concentration |
|----------------------------|---------------------|--------------|---------------------|---------------------|
| 2X Master Mix* | 2X | 2.5 | 296.88 | 1X |
| AbD primer/probe mix (80x) | 40x | .0625 | 6 | 0.5x |
| PCR-quality H2O | — | 2.44 | 234.24 | — |
| DNA (dried in 384) | 4.5 ng/µl | 4 | — | 3.6 ng/µl (18 ng) |
| Final Volume (µl) | | 5.00 | 357.44 | |

*The Master Mix was JUMPSTART™ Taq READYMIX™ (Sigma Catalogue No. 2893; Sigma Chemical Co., St. Louis, Missouri, United States of America), a premix of all the components, including nucleotides and Taq polymerase (but not primers and/or probes) necessary to perform a PCT reaction. Before use, 1375 µl of 1.0M MgCl₂ (Sigma Catalogue No. M1028) and 250 µl of 300 µM Sulforhodamine 101 (Sigma Catalogue No. S7635; ROX) were added to a 125 mL bottle of JUMPSTART™ Taq READYMIX™.

PCR plates were placed in an ABI 9700 thermal cycler and the following program was run: an initial denaturation of 50° C. for 2 minutes followed by 95° C. for 10 minutes; 40 cycles of 95° C. for 15 seconds/60° C. for 1 minute; and a final elongation of 72° C. for 5 minutes. After the cycling, the samples were incubated at 4° C. until needed.

The ABI 7900 Sequence Detection System (or TAQMAN®) was used to visualize the results of an allelic discrimination (SNP) assay. Using the Sequence Detection System (SDS) software, allele calls were made based on the fluorescence for the two dyes measured in each sample.

It will be understood that various details of the presently disclosed subject matter may be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

-continued

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attttgctgt gactgggtggg tctggcatat ttgagggtgt taaagggtcaa gtgaagctgc    180
gtcagattgt gtatcctttc aagatthttgt acacatttta tctaaagggt atcaaggatt    240
tgcctcagga gcttcttgtc aagactggtg agccaattcc atctgttgaa ccttcccctg    300
ctgctaaggc ccttgagccc aatgctacca ttgctggctt caccgactaa ttcatacaact    360
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tgcagttgcg ttagcatgtg aacaagctca tcaactagaag gcctatctgc aggcataatca    180
gagaggcatg cagcagcaat cctaaccgcc attagcatct catcttctc accttcttcc    240
cctaacaatgc tcttrtctag agcttctstgc gcctcgcccg cttgctgcaa gtgtctcagc    300
caacatocca aacttcccc actggctgct tctccaaaga atggatctgt aggatcctta    360
ccagttaaca aaacacctag tatcatgcca aaactaaaga tgtcactttt gtcagtgtac    420
ctgctgaaat tgccaaaaaa caccattcaa tctgaatacc atgaagctcc acatatacaa    480
gaatggaatg aagthaaaat aaacatctta ttggcaatac cataccata tatgcaatgc    540
catcaatcaa gcaatctttt attgthatta cttattgcta ttgatattga tgcattcaga    600
tatacaccac tgaaaaacta gaagtattcc aagthaaatt aggaaaaaaa acctthgtca    660
attattatta taatttagtt gtggtctcac tcaccaatct aggtthtagta gthtgcagca    720
tgtgaaactat aaactatatt attcatttgg accagactta gtgccaactg cctaaggctct    780
aaaacttgac atcaggaagt aatgggtagt tacaaaaaca aatagcaaag tctthattaa    840
ataataccaa attcaatcag caaatgatac aatgacaata cagattacag aatartaatt    900
tcgthtcaaa acaagagtct ttcattagggt trctacaaag ggggcaaaaa accaatgagg    960
tgtggagctt ttgaaaagggt aacgaatcaa acaattccc aatatcaacc acctaattag    1020
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<223> OTHER INFORMATION: n is a or g or c or t

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ttttctgttg ctttgcacat tgacatgcat ggtgagaaac ttaataaatt tcccttrgac    180
aycaaaggat actactgtgg tgagaggagc actgaagagc ttggagtagg tgggtgcagtt    240
gtgtaccaaa catttgttca aggctgcat ggtagtgcac ctctaggtc tattctgaaa    300
tgctgctgga gtgaggacga gaacgaagat gaagtgaggg agatataagt acttctggtt    360
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ctatcattaa acaatttagt ctataccacc tatgccccaa tagatataga tatatagaaa    180
caaaccaggt ggaagaggaa taacagagaa gtrtataaaa taaatatgca acttcagact    240
tgtatgctca agccgcaagc ttatatttga agatgtctat cccacaattc cagatccacc    300
taaacaagaa taaaatcca caaacatta gaagcgaatt cgctgctgca aattagaata    360
taaaattctt aaaaataatc cttacccaaa gattcatcac attaataata gtatccatgc    420
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atctcaatct gctttgacag atagagagaa ggagaatctt gacaacttag ttgacaagga    60
gtgtgcaacm acagagggta tgatatcgag ccagctcaag ttgaagtctt ctttgatc    120
caaagcgtct tctcatcaaa gcatggacaa acatgcaatc cttcgacgta tcaggcaacg    180
caggtttaat aacaaagcca aaaccgctct ggaagctctc ataggcacct cagaagccaa    240
caataccggc actgcccaag aacagaagtg gcttcaactg ggtgactctt tctcatctcc    300
ttaactggaa acacaatctc cccccagttt gcaacccatc tataaatwcc ctctttagtt    360
tccttcaccc aacaaagtca tcttttccat acgcttgcac ggctaggaaa ggaaatccaa    420
cccgatcttt atcggcatgc ttaagaaatc ccactatgta tctattaact gtagtagacc    480
tggtcggtt                                                                    489

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<210> SEQ ID NO 6
<211> LENGTH: 794
<212> TYPE: DNA
<213> ORGANISM: Glycine max
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(794)
<223> OTHER INFORMATION: r is a or g
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(794)
<223> OTHER INFORMATION: w is a or t

```

```

<400> SEQUENCE: 6

```

```

ccaaccacgc tgattcttta ggccatcagt caacccttta ctctggaaac tgctaaggaa    60
tataaacctt gaaccagaca tgtcccctga agagtagaaa aatatcatgt ttcagcagca    120
tggttcaaga aaatttgctc atgtaggcaa aattgatttt gaaagaaatt gattatgcaa    180
aattaatggt ggtaagatt aattttaaag taatgtgatt tatgtttgaa tattttttat    240
cgtagcaact cagtataact tttttaattc aatgcaaaaa tattcaaagt tattttgact    300
caatcaattc tagattcaat atcaattcct catcattgaa acaaatccca acatgtatct    360
aaaatcacga tagttgatat ttcaacgtaa ttcagaaaa atcaacataa aactaaccac    420
gcatcactta atgctgctga gggtagcat taaaattgat ttcaaataaa attaattttc    480
taaaattggt tttggtaaaa attgattttg aagtgcacatg agttatggtt gaacgttttt    540
tattctaaaa gttaataaca aatttcaawt tttttttatc caaagcaaaa gtcactctaaa    600
agttatttga actcgaaatc aaatttaaac tcaaaatcaa ttccttaatg tgaaatctaaa    660
catgtgtcca tttacctcaa atcatgttat ccgatatttc aacaagactt taaaraatcc    720
tacgtgaaac caaacatgca cttactagaa taagcagagt gaatagcaga aataccaga    780
ttgtaagctg caga                                                                    794

```

```

<210> SEQ ID NO 7
<211> LENGTH: 568
<212> TYPE: DNA
<213> ORGANISM: Glycine max
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(568)
<223> OTHER INFORMATION: r is a or g
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(568)
<223> OTHER INFORMATION: w is a or t

```

```

<400> SEQUENCE: 7

```

```

gttttacaca gttgcacaaa gtcataaact tcagtaccca ttttttatat tagcctggca    60

```

-continued

```

gtaagatggc tatttatttc tgagtgtagt ttgtactgat ttaatggttt tattatatgc 120
ctagactttt ctttgatata aatatcttat gtcatcttct cttggttgtg gatggatttt 180
ttcttctgtg gtgtawtttg ttaaaccaat gatattgtat attaattact tttgtaattt 240
tgatctagta atctattttc atggctattt taaaatattg tagtgggttg aggggaaaat 300
atthagggaa attatttagg ttgactgtgc cttgcctttt aggaatgcat aacgactttt 360
tttcttctaa cagttgratt atatacttag ctttgtaatt aattttgttt arttattcaa 420
ccgatttttt tcccacttta ttagcattta tcatgatctg gttccttcac tatattttgt 480
ttctgctagt gccaattttt tacctcaaga ctttactttt gtagcttttg tgctgtttat 540
ttgtttctgt ggatttgtct cgattcat 568

```

```

<210> SEQ ID NO 8
<211> LENGTH: 503
<212> TYPE: DNA
<213> ORGANISM: Glycine max
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(503)
<223> OTHER INFORMATION: r is a or g
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(503)
<223> OTHER INFORMATION: w is a or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(503)
<223> OTHER INFORMATION: y is c or t

```

```

<400> SEQUENCE: 8

```

```

ccgagacgrg tgaggaaatc ctgaggtgg gtcaaggacc cctcgtgctt gcttgggtcg 60
aacatggagg caacgagagt acaaatggtt agggacacar taagctggtt ttygaggaac 120
tagtacctcc agcaattcaa taaagaagaa gaagaacaaa gagggaaaga gtgaggtaat 180
tggaaatttc attcatgcct tgcttgttat ttacattagt atttataaac ttctagtaa 240
cagaaagatc ctaggaatat ttgccataa caaaatttgt acacattgtc ccctaggagc 300
agacaatata aaccaaggaa attctagaag gtagcctcag ctggctcac cwtcttcca 360
aaaacgtact ctttraggta agaaggtttt gtaattcttc tttegaactt agcttcttcc 420
tgtacccctt catttgcttt tgtgaccctt gccttgctt ctgtttgtaa gctcgtatca 480
acatrtttcc gatacagatg cag 503

```

```

<210> SEQ ID NO 9
<211> LENGTH: 769
<212> TYPE: DNA
<213> ORGANISM: Glycine max
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(769)
<223> OTHER INFORMATION: m is a or c
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(769)
<223> OTHER INFORMATION: r is a or g
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(769)
<223> OTHER INFORMATION: s is c or g
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(769)
<223> OTHER INFORMATION: w is a or t
<220> FEATURE:

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<221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(769)
 <223> OTHER INFORMATION: y i s c o r t

<400> SEQUENCE: 9

```

aacagagttg gctataacaa ggttttggat aatgccaagc ccatgaatga tccagatgga      60
aggcactttt catcttttac ttacctgagg cttagttcac ttctcatgga acgacaaaac      120
ttcatagagt ttgaaagatt tgtcaaraga atgcatggta agttgatgaa gaaaatttta      180
aatgtatcty tagcctctaa ttgatttatg aaaatatcta ttaaggaaag aaaatcatcc      240
ctttatctgg ccttgattga atgaatgctc acgtgtcadc tgraaccttc ttactccaca      300
gtcccacact cagtagggta aatgggtaat carcaaaaacc ctgaagacc aaatttcttt      360
cagaggcatg gttcagcaac tgtactaata tcatattgaa ctttgattc cattattcgm      420
tcccactgat agtgttcttt gatccacctw gcactagtct ttttatctga ttggaatcat      480
tttataaata attgaacacg aaasgagtat taatgtcagt ttttaagaggt gtacttctct      540
tggagagaaa aacctgaaa ctttaagtag ctgcaaaagt tgttgctatc ttgtgggaca      600
ttgattatat tagggttgat gactgtgtca tttctcattg caggggaagc tgttcttgat      660
cttcagggat aaccggtggg aaacaaagtc ttgttgaatt tgtatttata agtcccctga      720
caaaatgggt gggggattct gttatagaat aggagaaata ggattgtta      769

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<210> SEQ ID NO 10
 <211> LENGTH: 513
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(513)
 <223> OTHER INFORMATION: m i s a o r c
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(513)
 <223> OTHER INFORMATION: r i s a o r g

<400> SEQUENCE: 10

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gagaaagaga agtattgagc atcatgcca aatgtggcca cacttttcat ctttcttgca      60
ttgatatatg gctgaggaaa caatccacct gtccagtatg ccgtctgccg ttgaaaaact      120
cttccgaaac gaaacatgtg agacctgtga catttaccat gagccaatcc cttgacgagt      180
ctcacacatc agacagaaac gatgmtattg agagatatgt tgaacctaca cctactgcag      240
ccagtaactc tttacaacca acttcaggag aacaagaagc aagrcaatga tcttagagaa      300
ctaaaggggt tgttctgctc aaaaagagaa gaatgtagaa tttctgcttc tatagaggaa      360
tgcttctaata tatagattgg attcaaattc tttgtctgta atatggcctt catattcact      420
tggtgggtgta aatatgtttc cttttgtagc atatgcgggc caaggttttg gtggaatttc      480
ttgcataccg atttgaagtt cttttgtcta tgg      513

```

<210> SEQ ID NO 11
 <211> LENGTH: 281
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(281)
 <223> OTHER INFORMATION: y i s c o r t

<400> SEQUENCE: 11

```

atccaccaac accacttcac caccaaagcc tagtggagct gtaactgttt ccagtggcat      60

```


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tggccttttg gtggggtcct tctttgtgtc agcaattatg tytggtttca tgggctaggg 120
aagaggcagt gctgtttttt tttttattct cttttttatt aatatgttat tgttttgctg 180
agagtttgtg tgaatttcat catttaatcc agatgtatat ctaatcttga tatgtatctc 240
cttttaatat ttaaaaatga cacatttctt tagctgcttc c 281

```

```

<210> SEQ ID NO 12
<211> LENGTH: 948
<212> TYPE: DNA
<213> ORGANISM: Glycine max
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(948)
<223> OTHER INFORMATION: w i s a o r t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(948)
<223> OTHER INFORMATION: n i s a o r g o r c o r t

```

```

<400> SEQUENCE: 12

```

```

tgaatTTTTG ttatgagagc atctccattt taaagagatt tcgtttctgg gtatcagaaa 60
acacctccct tccgattaga tttctatatt ttgcctagac gtcaaagca cgatgaattc 120
tttgatgat gtttgaat tgaagtcttc tgtaatacwt gattatcaac ctcaaatttt 180
gatgagcaaa caagttatgc tttgtgaant tgttattgat gattgtatgg tgtgatttgg 240
tttcagggtt gaaacttgtt actgtggacc gtccttttgc tgagaagcac tatgctgatt 300
tgtctgcaaa gcctttcttt agtggtttgg tggattacat tatctctggc cctgttgttg 360
ccatgatctg ggagggcaag aatggttcta caactggccg aaagattatn ggagccacaa 420
accagctca atctgagcct ggaactatcc gtggtgactt tgccattgac attggaaggt 480
atagacttgt tttcatcatc gttcaagcaa gtttacgttt tcaaactctt tgttttaaca 540
catgaatatg aatctgtttt cattgttgtt catgtttcta atctagtttg gtggatgtta 600
ggttttcatt nttgttgtt gattttcaat ttctccgaa ttttttctt atttacaaca 660
ttggtttcca acgttctgtg ttttagagaa aagagtgttg tgctcaacca attctgcaaa 720
tattgcaata tgttcatgac atcatcaatt aagcaagatg ataactgt tgagtacatc 780
ataatcaagg gagtgaatc caacaaatcc atgttctgta tcttgattc atgaaaatca 840
aaattgttgg ggtagtcaat gtttatcaac attaaaaggt agtgtgnatc tanatactta 900
ttatttncaa ttctataaag gatttgaagg gaactttgaa tttttttt 948

```

```

<210> SEQ ID NO 13
<211> LENGTH: 485
<212> TYPE: DNA
<213> ORGANISM: Glycine max
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(485)
<223> OTHER INFORMATION: r i s a o r g

```

```

<400> SEQUENCE: 13

```

```

ttctatgacc aacctaccgc ctgtgggtaa agctaaacta agttctcaca ggtgaggtga 60
gttcaccaac cacggccgcc agaatttcca gatccccatc cgttccctga tgctgctttc 120
ttgggggcag atccccagcc agagcttctt tgttcaccat cacttggacc agcaccacca 180
ctcgttgtc cccccagcc accattgtca ctgttactag ctccactcc tccccctccc 240
caaccactac tgctccacc gttccacca ccaccacccc aaccaccagg aaaagcttcc 300

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cttccaggag aattctgaac cttggcccct ggaaaattgc ttaaaccatc atccgartcc 360
 tttgtattat tagaacccca tctgcccaca taaccagagt cctgcctttc attattggag 420
 ttgtctctc tattgttata agaacctcga ccacgaccac gtacacgccc ttttccaccc 480
 accga 485

<210> SEQ ID NO 14
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 14
 aggatttgcc tcaggagctt 20

<210> SEQ ID NO 15
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 15
 gaaactccac cactacaaaa cc 22

<210> SEQ ID NO 16
 <211> LENGTH: 16
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 16
 agtggggact atgatg 16

<210> SEQ ID NO 17
 <211> LENGTH: 17
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 17
 agtgggaact atgatgg 17

<210> SEQ ID NO 18
 <211> LENGTH: 31
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 18
 caatcagcaa atgatacaat gacaatacag a 31

<210> SEQ ID NO 19
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 19
 agctccacac ctcattgggtt tt 22

<210> SEQ ID NO 20
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 20
 tttcattagg gtgctacaa 19

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<210> SEQ ID NO 21
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 21

 tctttcatta gggactaca a 21

<210> SEQ ID NO 22
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 22

 caaaagctcc acacctcatt gg 22

<210> SEQ ID NO 23
 <211> LENGTH: 25
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 23

 ttgacatcag gaagtaatgg gtagt 25

<210> SEQ ID NO 24
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 24

 ttgaaacgaa attattattc tg 22

<210> SEQ ID NO 25
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 25

 tgttttgaaa cgaaattact a 21

<210> SEQ ID NO 26
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 26

 gggactcagt tacgcaagtt ttg 23

<210> SEQ ID NO 27
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 27

 gtgctcctct caccacagta g 21

<210> SEQ ID NO 28
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 28

 aagctggtgg ttatgattt 19

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<210> SEQ ID NO 29
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 29

 agctgttggt tatgactt 18

<210> SEQ ID NO 30
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 30

 atcccacaat tccagatcca 20

<210> SEQ ID NO 31
 <211> LENGTH: 26
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 31

 ttatggtaga ggttcatatt catctt 26

<210> SEQ ID NO 32
 <211> LENGTH: 16
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 32

 agccatcacc cccgta 16

<210> SEQ ID NO 33
 <211> LENGTH: 16
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 33

 agccatcacc cccata 16

<210> SEQ ID NO 34
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 34

 agtctatacc acctatgccc aaa 23

<210> SEQ ID NO 35
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 35

 ggcttgagca tacaagtctg aa 22

<210> SEQ ID NO 36
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

 <400> SEQUENCE: 36

 aggaataaca gagaagtgta 20

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<210> SEQ ID NO 37
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 37
aggaataaca gagaagtata ta 22

<210> SEQ ID NO 38
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 38
agagagaagg agaatcttga caact 25

<210> SEQ ID NO 39
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 39
acgtcgaagg attgcatggt tg 22

<210> SEQ ID NO 40
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 40
aggagtgtgc aaccac 16

<210> SEQ ID NO 41
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 41
aggagtgtgc aacaaca 17

<210> SEQ ID NO 42
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 42
ccccagtttg caaccatct 20

<210> SEQ ID NO 43
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 43
ccatgcaagc gtatgaaaa gat 23

<210> SEQ ID NO 44
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 44

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| | |
|--|----|
| ctaaagaggg aatttat | 17 |
| <210> SEQ ID NO 45 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 45 | |
| aaactaaaga gggatattat | 20 |
| <210> SEQ ID NO 46 <211> LENGTH: 29 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 46 | |
| gtgtccattt acctcaaadc atgttatcc | 29 |
| <210> SEQ ID NO 47 <211> LENGTH: 24 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 47 | |
| gtaagtgcac gtttggtttc acgt | 24 |
| <210> SEQ ID NO 48 <211> LENGTH: 18 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 48 | |
| aagactttaa agaadcct | 18 |
| <210> SEQ ID NO 49 <211> LENGTH: 19 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 49 | |
| caagacttta aaaaadcct | 19 |
| <210> SEQ ID NO 50 <211> LENGTH: 22 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 50 | |
| taaatgctaa taaagtggga aa | 22 |
| <210> SEQ ID NO 51 <211> LENGTH: 16 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 51 | |
| aggttgactg tgcctt | 16 |
| <210> SEQ ID NO 52 <211> LENGTH: 21 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 52 | |

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| | |
|--|----|
| agctaagtat ataatccaac t | 21 |
| <210> SEQ ID NO 53 <211> LENGTH: 22 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 53 | |
| agctaagtat ataattcaac tg | 22 |
| <210> SEQ ID NO 54 <211> LENGTH: 25 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 54 | |
| ggaggcaacg agagtacaaa atggt | 25 |
| <210> SEQ ID NO 55 <211> LENGTH: 25 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 55 | |
| tgaattgctg gaggtactag ttcct | 25 |
| <210> SEQ ID NO 56 <211> LENGTH: 16 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 56 | |
| cagcttactg tgtccc | 16 |
| <210> SEQ ID NO 57 <211> LENGTH: 17 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 57 | |
| ccagcttatt gtgtccc | 17 |
| <210> SEQ ID NO 58 <211> LENGTH: 22 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 58 | |
| gaacatggag gcaacgagag ta | 22 |
| <210> SEQ ID NO 59 <211> LENGTH: 29 <212> TYPE: DNA <213> ORGANISM: Glycine max <400> SEQUENCE: 59 | |
| ttcttcttta ttgaattgct ggaggtact | 29 |
| <210> SEQ ID NO 60 <211> LENGTH: 16 <212> TYPE: DNA <213> ORGANISM: Glycine max | |

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<400> SEQUENCE: 60
 ttctcaaaa accagc 16

<210> SEQ ID NO 61
 <211> LENGTH: 15
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 61
 tcctcgaaaa ccagc 15

<210> SEQ ID NO 62
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 62
 tttcataaat caattagagg cta 23

<210> SEQ ID NO 63
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 63
 gaggcttagt tcacttctc 19

<210> SEQ ID NO 64
 <211> LENGTH: 16
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 64
 accatgcatt ctcttg 16

<210> SEQ ID NO 65
 <211> LENGTH: 17
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 65
 accatgcatt cttttga 17

<210> SEQ ID NO 66
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 66
 tcccttgacg agtctcacac at 22

<210> SEQ ID NO 67
 <211> LENGTH: 24
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 67
 ttgcttcttg ttctcctgaa gttg 24

<210> SEQ ID NO 68
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

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<400> SEQUENCE: 68
cagacagaaa cgatgata 18

<210> SEQ ID NO 69
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 69
cagaaacgat gctattg 17

<210> SEQ ID NO 70
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 70
gtggcattgg ccttttgg 19

<210> SEQ ID NO 71
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 71
cactgcctct tccctagcc 19

<210> SEQ ID NO 72
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 72
catgaaacca aacataat 18

<210> SEQ ID NO 73
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 73
catgaaacca gacataat 18

<210> SEQ ID NO 74
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 74
gcacgatgaa ttctttgtat gatgtttga 29

<210> SEQ ID NO 75
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 75
aaagcataac ttgttgtegc atcaa 25

<210> SEQ ID NO 76
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Glycine max

<400> SEQUENCE: 76

ttctgtaata cttgattatc 20

<210> SEQ ID NO 77

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 77

ttctgtaata catgattatc 20

<210> SEQ ID NO 78

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 78

tcccttccag gagaattctg aac 23

<210> SEQ ID NO 79

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 79

tgaaaggcag gactctggtt atg 23

<210> SEQ ID NO 80

<211> LENGTH: 17

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 80

aaccatcatc cgaatcc 17

<210> SEQ ID NO 81

<211> LENGTH: 16

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 81

taaaccatca tccgag 16

<210> SEQ ID NO 82

<211> LENGTH: 459

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 82

ttttgatcca aaacaaagct gaaaagaaag gggacaggta tgaagcaatc ttcagcttct 60

actttggaga ctatggtcac atagcagtgc agggacctta cctgacctat gaggacacat 120

atttggetgt gactgggtggg tctggcatat ttgagggtgt taaagggtcaa gtgaagctgc 180

gtcagattgt gtatcctttc aagatthttgt acacatttta tctaaagggt atcaaggatt 240

tgctcagga gcttcttgtc aagactggtg agccaattcc atctggtgaa ccttcccctg 300

ctgctaaggc ccttgagccc aatgctacca ttgctggctt caccgactaa ttcatacaact 360

ttttttgtat ttgctttggc cttttagtagta gtatgattta agttactgaa taataataac 420

aagtggggac tatgatgggt tttgtagtgg tggagtttc 459

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<210> SEQ ID NO 83
 <211> LENGTH: 1102
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 83

```

ttacaccaga atcatggcca ccaatcaa ataccctatca tcttattaca gaaaagggga      60
taagagaagg ggaaaaaaaa tctctagaaa ctgaagaatc aaggtttggg tcaaaaactg    120
tgcagttgcg ttagcatgtg aacaagctca tcaactagaag gcctatctgc aggcatatca    180
gagaggcatg cagcagcaat cctaaccgcc attagcatct catcttctct accttcttcc    240
cctaacatgc tcttatctag agcttctgtg gcctcgcccc cttgctgcaa gtgtctcagc    300
caacatocca aacttcccc actggctgct tctccaaaga atggatctgt aggatcctta    360
ccagttaaca aacacctag tatcatgcca aaactaaaga tgtcactttt gtcagtgtac    420
ctgctgaaat tgccaaaaaa caccattcaa tctgaatacc atgaagctcc acatatacaa    480
gaatggaatg aagttaaaat aaacatctta ttggcaatac cataccata tatgcaatgc    540
catcaatcaa gcaatctttt attggtatta cttattgcta ttgatattga tgcacccaaa    600
tatacaccac tgaaaaacta gaagtattcc aagttaaatt aggaaaaaaa acctttgtca    660
attattatta taatttagtt gtggtctcac tcaccaatct aggttttagta gtttgcagca    720
tgtgaactat aaactatatt attcatttgg accagactta gtgccaaactg cctaaggtct    780
aaaacttgac atcaggaagt aatgggtagt taaaaaaca aatagcaaag tctttattaa    840
ataataccaa attcaatcag caaatgatac aatgacaata cagattacag aatagtaatt    900
tcgtttcaaa acaagagtct ttcattaggg tactacaaag ggggcaaaaa accaatgagg    960
tgtggagctt ttgaaaagg aacgaatcaa acaaatccc aatatcaacc acctaattac   1020
cctattggtg taaaaaagtg aaagaacaga aacagacca gttcaaaaca ctagtgaatg   1080
agtatgattg aaatttgtct ta                                     1102
  
```

<210> SEQ ID NO 84
 <211> LENGTH: 402
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 84

```

tcagttacgc aagttttgaa gctggtgggt atgactttaa tgacatggct ctaggtgaac      60
ttgtggaaag gatttttagct tgcttttctc cagcagagtt ttctgttctt ttgcacattg    120
acatgcatgg tgagaaactt aataaatttc ccttagacat caaaggatac tactgtggtg    180
agaggagcac tgaagagctt ggagtaggtg gtgcagttgt gtaccaaaaca tttgttcaag    240
gctgcatgg tagtgcctc cctaggtcta ttctgaaatg ctgctggagt gaggacgaga    300
acgaagatga agtgaggag atataagtac ttctggtttt tatgttctgt tgttatttat    360
ctttactgtc gagtgtgtta ttacctaat aaataaatat gg                                     402
  
```

<210> SEQ ID NO 85
 <211> LENGTH: 466
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 85

```

atcctcaaat acaagtcctt gatctcatca tacctaagtt gttaaagaaa atctagccca      60
  
```

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| | |
|--|-----|
| ttccatataa atcttattaa taccttactc caggacctga aagacaacct acccaactat | 120 |
| cattaaaciaa tttagtctat accacctatg cccaaataga tatagatata tagaaacaaa | 180 |
| ccaggtggaa gaggaataac agagaagtgt ataaaataaa tatgcaactt cagacttgta | 240 |
| tgctcaagcc gcaagcttat atttgaagat gtctatccca caattccaga tccacctaaa | 300 |
| caagaataaa aatccacaaa acattagaag cgaattcgct gctgcaaatt agaataataa | 360 |
| attcttaaaa ataatcctta ccaaaagatt catcacatta ataatagtat ccatgcccat | 420 |
| gtgagccatc acccccgtaa taagatgaat atgaacctct accata | 466 |

<210> SEQ ID NO 86
 <211> LENGTH: 489
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 86

| | |
|---|-----|
| atctcaatct gctttgacag atagagagaa ggagaatctt gacaacttag ttgacaagga | 60 |
| gtgtgcaacc acagagggtg tgatctcgag ccagctcaag ttgaagtctt ctttgctatc | 120 |
| caaagcgtct tctcatcaaa gcatggacaa acatgcaatc cttcgacgta tcaggcaacg | 180 |
| caggtttaat aacaaagcca aaaccgctct ggaagctctc ataggcacct cagaagccaa | 240 |
| caataccggc actgcccaag aacagaagtg gcttcaactg ggtgactctt tctcatctcc | 300 |
| ttaactggaa acacaatctc cccccagttt gcaacccatc tataaatacc ctctttagtt | 360 |
| tccttcaccc aacaaagtca tcttttccat acgcttgcat ggctaggaaa ggaaatccaa | 420 |
| cccgatcttt atcggcatgc ttaagaaatc cactatgta tctattaact gtagtagacc | 480 |
| ttgtcggtt | 489 |

<210> SEQ ID NO 87
 <211> LENGTH: 794
 <212> TYPE: DNA
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 87

| | |
|--|-----|
| ccaaccacgc tgattcttta ggccatcagt caacaaatta ctctggaaac tgctaaggaa | 60 |
| tataaacctt gaaccagaca tgtcccctga agagtagaaa aatatcatgt ttcagcagca | 120 |
| tggttcaaga aaatttggtc atgtaggcaa aattgatctt gaaagaaatt gattatgcaa | 180 |
| aattaatggt ggtaagatt aattttaaag taatgtgatt tatgtttgaa tattttttat | 240 |
| cgtagcaact cagtataact tttttaattc aatgcaaaaa tattcaaagt tattttgact | 300 |
| caatcaattc tagattcaat atcaattcct catcattgaa acaaatgcca acatgtatct | 360 |
| aaaatcacga tagttgatat ttcaacgtaa ttcaagaaaa ttcaacataa aactaaacac | 420 |
| gcatcactta atgctgctgta gggtagcat taaaattgat ttcaataaaa attaatcttc | 480 |
| taaaattggt tttggtaaaa attgattttg aagtgcacatg agttatggtt gaacgttttt | 540 |
| tattctaaaa gtttaataca aatttcaaat tttttttatc caaagcaaaa gtcattctaa | 600 |
| agttatttga actcgaatc aaatttaaac tcaaaatcaa ttccttaatg tgaaattaaa | 660 |
| catgtgtcca tttacctcaa atcatgttat ccgatatttc aacaagactt taagaatcc | 720 |
| tacgtgaaac caaacatgca cttactagaa taagcagagt gaatagcaga aataccaga | 780 |
| ttgtaagctg caga | 794 |

<210> SEQ ID NO 88
 <211> LENGTH: 568

-continued

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 88

```

atgaatagag acaaatccac agaaacaaat aaacagcaca aaagctacaa aagtaaagtc    60
ttgaggtaaa aaattggcac tagcagaaac aaaatatagt gaaggaacca gatcatgata    120
aatgctaata aagtgggaaa aaaatcgggt gaataattaa acaaaattaa ttacaaagct    180
aagtatataa ttcaactggt agaagaaaaa aagtcggtat gcattcctaa aaggcaaggc    240
acagtcaacc taaataattt ccctaaatat tttcccctca acccactaca atattttaaa    300
atagccatga aaatagatta ctagatcaaa attacaaaag taattaatat acaatatcat    360
tggtttaaca aattacacca cagaagaaaa ataccatcca caaccaagag aagatgacat    420
aagatattga tatcaaagaa aagtctaggc atataataaa accattaaat cagtacaaac    480
tacctcaga aataaatagc catcttactg ccaggctaat ataaaaaatg gtcactgaag    540
tttatgactt tgtgcaactg tgtaaaac                                     568

```

<210> SEQ ID NO 89

<211> LENGTH: 503

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 89

```

ccgagacgag tgaggaaatc ctcgaggtgg gtcaaggacc cctcgtgctt gcttgggtcg    60
aacatggagg caacgagagt acaaaatggt agggacacaa taagctgggt ttcgaggaac    120
tagtacctcc agcaattcaa taaagaagaa gaagaacaaa gagggaaaga gtgaggtaat    180
tggaaatttc attcatgcct tgcttggtat ttacattagt atttataaac ttactagtaa    240
cagaaagatc ctaggaatat ttgccataa caaaatttgt acacattgtc ccctaggagc    300
agacaatata aaccaaggaa attctagaag gtagcctcag ctggtctcac ccttcttcca    360
aaaacgtact ctttgaggta agaaggtttt gtaattcttc tttcgaactt agcttcttcc    420
tgtacccctt catttgcttt tgtgaccctt gcccttgctt ctgtttgtaa gctcgtatca    480
acatgtttcc gatacagatg cag                                     503

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<210> SEQ ID NO 90

<211> LENGTH: 762

<212> TYPE: DNA

<213> ORGANISM: Glycine max

<400> SEQUENCE: 90

```

aacagagttg gctataacaa ggttttggat aatgccaaagc ccatgaatga tccagatgga    60
aggcactttt catcttttac ttacctgagg cttagttcac ttctcatgga acgacaaaac    120
ttcatagagt ttgaaagatt tgtcaagaga atgcatggta agttgatgaa gaaaatttta    180
aatgtatctc tagcctctaa ttgatttatg aaaatatcta ttaaggaaag aaaatcatcc    240
ctttatctgg ccttgattga atgaatgctc acgtgtcctc tgaaaccttc ttactccaca    300
gtcccacact cagtagggtg atcagcaaaa ccctgaagac ccaaatttct ttcagaggca    360
tggttcagca actgtactaa tatcatattg aactttggat tccattattc gctcccactg    420
atagtgttct ttgatccacc tagcactagt ctttttatct gattggaatc attttataaa    480
taattgaaca cgaaaggagt attaatgtca gttttaagag gtgtacttct cttggagaga    540
aaaacactga aactttaagt agctgcaaaa gttgttgcta tcttgtggga cattgattat    600

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attagggttg atgactgtgt catttctcat tgcaggggaa gctgttcttg atcttcaggt 660
ataaccgggtg ggaacaaaag tcttggtgaa tttgtattta taagtcccct gacaaaatgg 720
ttgggggtat tctgttatag aataggagaa ataggattgt ta 762

```

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<210> SEQ ID NO 91
<211> LENGTH: 513
<212> TYPE: DNA
<213> ORGANISM: Glycine max

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<400> SEQUENCE: 91

```

```

gagaaagaga agtattgctc atcatgccc aatgtggcca cacttttcat ctttcttgca 60
ttgatatatg gctgaggaaa caatccacct gtccagtatg cctgtctgcc ttgaaaaact 120
cttccgaaac gaaacatgtg agacctgtga cattaccat gagccaatcc cttgacgagt 180
ctcacacatc agacagaaac gatgatattg agagatatgt tgaacctaca cctactgcag 240
ccagtaactc tttacaacca acttcaggag aacaagaagc aaggcaatga tcttagagaa 300
ctaaaggggt tgttctgctc aaaaagagaa gaatgtagaa tttctgctc tatagaggaa 360
tgcttcta at tatagattgg attcaaattc tttgtctgta atatggcctt catattcact 420
tggtggtgta aatatgtttc cttttgtagc atatgcccgc caaggttttg gtggaatttc 480
ttgcataccg atttgaagtt cttttgtcta tgg 513

```

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<210> SEQ ID NO 92
<211> LENGTH: 280
<212> TYPE: DNA
<213> ORGANISM: Glycine max

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<400> SEQUENCE: 92

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```

atccaccaac accacttcac caccaaagcc tagtggagct gtaactgttt ccagtggcat 60
tggccttttg gtgggtctt tctttgtgtc agcaattatg tttggtttca tgggctaggg 120
aagaggcagt gctgtttttt ttttattctc ctttttatta atatgttatt gttttgctga 180
gagtttgtgt gaatttcac atttaatcca gatgtatatac taatcttgat atgtatctcc 240
ttttaatatt taaaaatgac acatttcttt agctgcttcc 280

```

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<210> SEQ ID NO 93
<211> LENGTH: 883
<212> TYPE: DNA
<213> ORGANISM: Glycine max

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<400> SEQUENCE: 93

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agatttcggt tctgggtatc agaaaacacc taggttccga ttagatttct atattttgcc 60
tagacgtcaa atgcacgatg aattctttgt atgatgtttg aaaattgaag tcttctgtaa 120
tacttgatta tcaacctcaa attttgatgc gacaacaagt tatgctttgt gaagttgtta 180
ttgatgattg tatgggtgta tttggtttca ggtttgaaac ttgttactgt ggaccgtcct 240
tttctgaga agcactatgc tgatttgtct gccaaagcctt tctttagtgg tttgggtggat 300
tacattatct ctggccctgt tgttgccatg atctgggagg gcaagaatgt tgttacaact 360
ggccgaaaga ttatcggagc cacaaacca gctcaatctg agcctggaac tatccgtggg 420
gactttgcca ttgacattgg aaggataga cttgttttca tcatcgttca agcaagtta 480
cgttttcaaa tcttttgttt taacacatga atatgaatct gttttcattg ttgttcatgt 540
ttctaatacta gtttgggtgga tgtttaggttt tcatttttgt tgtttgattt tcaatttctc 600
ccgaattttt ttcttattta caacattggt ttccaacggt ctgtgtttta gagaaaagag 660

```


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tgttgtgctc aaccaattct gcgaaatattg caatatgttc atgacatcat caattaagca 720
agatgataac actgttgagt acatcataat caaggcagtg gaatccaaac aaatccatgt 780
tctgtatctt tgtattcatg aaaatcaaaa attgttgttt tagtcaatgt ttatcaacat 840
tgctcttagt gtggatctag atacttatta ttacaatcc tat 883

```

```

<210> SEQ ID NO 94
<211> LENGTH: 475
<212> TYPE: DNA
<213> ORGANISM: Glycine max

```

```

<400> SEQUENCE: 94

```

```

atgaccaacc taccgcctgt gggtaaagct aaactaagtt ctcacaggtg aggtgagttc 60
accaaccacg gccgccagaa tttccagatc cccatccggt ccctgatgct gctttcttgg 120
gggcagatcc ccagccagag cttccttggt caccatcact tggaccagca ccaccactcg 180
ctgtceccc ccagccacca ttgtcactgt tactagctcc acctcctccc cctcccac 240
cactactgcc tccaccgctt ccaccaccac caccacaacc accaggaaaa gcttcccttc 300
caggagaatt ctgaaccttg gcccctggaa aattgcttaa accatcatcc gagtcctttg 360
tattattaga accccatctg ccccataac cagagtctcg ctttccatta ttggagttgt 420
ctcctctatt gttataagaa cctcgaccac gaccacgtcc acgcctctt ccacc 475

```

What is claimed is:

1. A method for producing a soybean rust (SBR) resistant soybean plant, the method comprising the steps of:

- (a) isolating one or more nucleic acids from a plurality of soybean plants;
- (b) genotyping said one or more nucleic acid for the presence of a marker associated with SBR resistance located within a chromosome interval, wherein said chromosome interval is located on soybean LG N and is defined by and comprises map positions 34.26 cM to 38.8 cM and wherein said chromosome interval further comprises at least one of an A at position 102 at SEQ ID NO: 11; a T at position 159 of SEQ ID NO: 12; and a G at position 357 of SEQ ID NO: 13;
- (c) selecting a first soybean plant on the basis of the presence of the marker genotyped in (b);
- (d) crossing the first soybean plant of (c) with a second soybean plant not having said chromosome interval;
- (e) collecting seed from the cross of (d); and
- (f) growing a progeny soybean plant from the seed of (e), wherein said progeny soybean plant comprises in its genome said chromosome interval thereby producing a SBR resistant soybean plant.

2. The method of claim 1, wherein the marker detected in (c) comprises an A at position 102 at SEQ ID NO: 11; a T at position 159 of SEQ ID NO: 12; and a G at position 357 of SEQ ID NO: 13.

30

3. The method of claim 1, wherein the genotyping in (b) comprises amplifying a marker locus or a portion of the marker locus and detecting the resulting amplified marker amplicon.

35

4. The method of claim 3, wherein the amplifying comprises: (a) admixing an amplification primer or amplification primer pair with a nucleic acid isolated from the first *Glycine max* plant or germplasm, wherein the primer or primer pair is complementary or partially complementary to at least a portion of the marker locus, and is capable of initiating DNA polymerization by a DNA polymerase using the soybean nucleic acid as a template; and (b) extending the primer or primer pair in a DNA polymerization reaction comprising a DNA polymerase and a template nucleic acid to generate at least one amplicon.

45

5. The method of claim 4, wherein the nucleic acid is selected from DNA and RNA.

50

6. The method of claim 4, wherein the amplifying comprises employing a polymerase chain reaction (PCR) or ligase chain reaction (LCR) using a nucleic acid isolated from the first soybean plant or germplasm as a template in the PCR or LCR.

55

7. The method of claim 6, wherein the amplifying is a PCR and the primers comprise any one of SEQ ID NOs: 70-81.

* * * * *